**PATENT**
**Attorney Docket No.:**
**053665-5005-02**

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



| | | |
|---|---|---|
| In re Application of: | | : |
| Louis S. KUCERA *et al.* | | : |
| Application No: | 09/412,539 | : Group Art Unit 1624 |
| Filed: | October 4, 1999 | : |
| Title: | LIPID ANALOGS FOR TREATING VIRAL INFECTIONS | : Examiner: Brenda Coleman |

## DECLARATION UNDER C.F.R. § 1.132

I hereby declare as follows:

1. I received a degree of Bachelor of Arts in Natural Science from St. John's University, Collegeville, Minnesota in 1957. I received a degree of Master of Science in Medical Microbiology from Creighton University, Omaha, Nebraska in 1959. I received a degree of Doctor of Philosophy in Medical Microbiology from University of Missouri, Columbia, Missouri in 1964.

2. I presently hold the position of Full Professor of Microbiology and Immunology at Wake Forest University School of Medicine, Winston-Salem, North Carolina, the assignee of the above-identified application, and Senior Vice-President/Founder of Kucera Pharmaceutical Company, Winston-Salem, North Carolina. I have been employed by Wake Forest University since 1970 and Kucera Pharmaceutical Company since 2001.

3. I am the author or co-author of over 130 publications, almost all of which pertain to antiviral agents or medical virology. I have also presented at numerous meetings, symposiums, workshops, and conferences and lectured at several universities.

4. My education, technical experience, professional activities, honors and awards, and list of publications are set forth in my *curriculum vitae* attached hereto as Exhibit A.

5. I am a co-inventor of the invention described and claimed in the above-identified application.

6. I am familiar with and understand the above-identified application ("Application"), including the pending claims and the outstanding rejections of the claims.

7. I and others have evaluated the activity of phospholipid-AZT conjugates against HIV. In our experiments, the lipid-AZT conjugates were synthesized by reacting 3-amino-1,2-propanediol with an acyl chloride to form an alkylamidopropanediol. The primary alcohol was protected as a trityl ether by reaction with trityl chloride and then the secondary alcohol was alkylated with an alkyl halide using sodium hydride as a base. After removing the trityl ether with p-toluenesulphonic acid, the hydroxy group was converted to a phosphatidic acid. The phosphatidic acid was coupled with AZT using dicyclohexylphosphodiimide as the condensing agent. The lipid-AZT conjugates were evaluated for cytoxicity, anti-HIV-1 activity and selectivity in CEM-SS and PBL cells. Further details of the materials and methods are set forth in Kucera *et al.* "*In vitro* evaluation characterization of newly designed alkylamidophospholipid analogues as anti-human immunodeficiency virus type 1 agents," Antiviral Chemistry & Chemotherapy 9:157-165 (1998) ("Kucera *et al.*"), attached hereto as Exhibit B.

8. The INK-14 compound described in Table 1 of Kucera *et al.* is a compound within the genus of Formula III of the specification of the Application.

9. The INK-14 compound gave a high selectivity index ("SI") of >1250 against infectious HIV-1 replication in CEM-SS cells, as shown in Table 2 of Kucera *et al.*

10. The specification commencing on page 14, line 20 through page 15, line 8 of the Application describes that experimentation has demonstrated efficacy of the compounds of Formula III.

11. The data in Kucera *et al.* demonstrates that the INK-14 compound shows clinical efficacy of Formula III compounds against HIV.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: April 30, 2002

Louis S. Kuccra, Ph.D.

Louis S. Kuccra, Ph.D.

WAKE FOREST UNIVERSITY
SCHOOL OF MEDICINE
CURRICULUM VITAE

Rev: 04-22-02



**NAME:** Louis Stephen Kucera, Ph.D.

**SOCIAL SECURITY NUMBER:** 469-34-4235

**ADDRESS:**

Residence: 4860 Ellen Avenue
Pfafftown, North Carolina 27040

Telephone: (336) 924-8702

Business: Department of Microbiology and Immunology
Wake Forest University School of Medicine
Medical Center Boulevard
Winston-Salem, North Carolina 27157-1064
Telephone: (910) 716-4875

**PERSONAL INFORMATION:**

Birthplace: New Prague, Minnesota

Birth Date: June 23, 1935

Citizenship: U.S.A.

Religion: Catholic

Marital Status: Married

Spouse's name: Jo Ann (11/2/37)

Children: Gregory (6/20/60)
Gary (12/31/61)
Stephen (1/23/63)
Scott (2/23/66)

**EDUCATION:**

1953-1957 St. John's University Collegeville, Minnesota B.A.

1960-1964 University of Missouri, Columbia, Missouri Ph.D.

**POSTDOCTORAL TRAINING:**

1964-1966 Mayo Clinic, Section of Microbiology, Rochester, MN, Applied Virology, Ernest C. Herrmann, Jr., Ph.D., Program Advisor

1966-1968 St. Jude Children's Research Hospital, Memphis, TN, Basic Virology, Allan Granoff, Ph.D., Program Advisor

**UNIFORMED SERVICE:**

Branch: Army Reserve
Dates Served: October 1959 - September 1961
Rank: Pfc
Awards: Honors, Southeastern Signal School

**EMPLOYMENT:**

1968-1970 Staff Member, Laboratory of Virology, St. Jude Children's Research Hospital, Memphis, TN

1968-1970 Assistant Professor, Department of Microbiology, University of Tennessee Medical School, Memphis, TN

1970-1974 Assistant Professor, Department of Microbiology and Immunology Bowman Gray School of Medicine of Wake Forest University Winston-Salem, N C

1974-1980 Associate Professor (tenured), Department of Microbiology and Immunology, Bowman Gray School of Medicine of Wake Forest University School of Medicine, Winston-Salem, NC

1983-1985 Professor and Acting Chairman Department of Microbiology and Immunology, Bowman Gray School of Medicine of Wake Forest University School of Medicine, Winston-Salem, NC

1980-present Full Professor (tenured) Department of Microbiology and Immunology, Wake Forest University School of Medicine, Winston-Salem, NC

2001-present Senior Vice President / Founder Kucera Pharmaceutical Company, Winston-Salem, NC 27101

**Courses Taught:**

Basic Virology
Advanced Topics in Virology
Medical Microbiology
Fundamental Techniques in Microbiology and Immunology
Advanced Topics in Microbiology, Immunology, and Virology
Biology of Neoplasia
Fundamantals of Virology
Molecular Pathogenesis of Cancer
Molecular Targets in Cancer Biology
Basic and Clinical Science Problems Course
Doctor-Patient Relationship Course
Medicine as a Profession

**Sponsorships:**

| | |
|---|---|
| 1970-1973 | Janice Simonson, M.S. |
| 1971-1974 | Phyllis Melvin M.S. |
| 1973-1975 | Suzanne M. Konopka, M.S. |
| 1975-1977 | Janice R. Connor, M.S. |
| 1973-1978 | Mario J. Marcon, Ph.D. |
| 1977-1981 | Richard A. Respess, Ph.D. |
| 1982-1984 | Richard T. Flanders, Ph.D. (Postdoctorate) |
| 1982-1986 | Catherine Krebs, Ph.D. |
| 1987-1989 | Nickolus Oldenburg (Undergraduate Student) |
| 1988-1989 | David Raben (Medical Student) |
| 1988-1989 | Malcolm Foster, III (Medical Student) |
| 1988-1989 | Adam Raben (Medical Student) |
| 1989 | Lisa Burman (Medical Student) |
| 1988-1990 | Girish Giridhar, Ph.D. (Postdoctorate) |
| 1987-1992 | Lisa Krugner-Higby, D.V.M. |
| 1990 | Vinette Edwards (Medical Student) |
| 1990 | David Goff (Medical Student) |
| 1990 | Jay Neufeld (Medical Student) |
| 1991 | Indu Anand (Medical Student) |
| 1991 | Christopher Philippart (Medical Student) |
| 1991 | Lewis McCurdy (Undergraduate Student, WFU) |
| 1991 | Laura Neese (Undergraduate Student, Salem College) |
| 1992 | William Thomas (Medical Student) |
| 1993 | Tianang Dai (Undergraduate Student, Salem College) |
| 1993-1995 | Brandi Bickford (Undergraduate Student, Salem College) |
| 1993 | Chris Whang (Medical Student) |

| | |
|---|---|
| 1993 | Cas Fowler (Medical Student) |
| 1994 | Jason Thompson (Undergraduate Student) |
| 1994 | Brian Whirrett (Undergraduate Student) |
| 1995 | Michael Hughes (Medical Student) |
| 1996 | Erica Humphrey (Undergraduate Student, Salem College) |
| 1996 | Christi Goff, (Undergraduate Student, Wake Forest University University) |
| 1996 | Harsha Setty (High School Student) |
| 1997 | Ina Shippy (High School Student) |
| 1997 | Zakery Zimmerman (Undergraduate Student, WFU) |
| 2000 | Jennifer Hu (Medical Student) |
| 2000 | Sherelle McCoy (CERTL High School Student) |
| 2001 | Daniel Leung (Medical Student) |
| 2002 | Ergaba Sheferaw (Salem College) |

**Intramural committee work:**

Member of Student Evaluation Committee
Microbiological Safety Committee
Administrative Assistant for Research Development and Controller of Biohazardous Agents, Department of Microbiology and Immunology, 1975-79
Program Director and Administrative Assistant for Graduate Student Affairs, 1980-83
Basic Science Task Group, Oncology Research Center
Medical Student Admissions Committee
Group Leader for Oncogenesis Affinity Group, Oncology Research Center, 1972-84
Intramural Research Committee
Member of the Oncology Research Center, 1970-present
Member, Executive Committee, Department of Microbiology and Immunology, 1974-
Medical Student Advisor, 1972-present
Director, Tissue Culture Core Laboratory, 1979-present
Coordinator, Viral Oncology and Tumor Immunology Seminar Series
Biomedical Graduate Committee Member, 1980-85
Graduate Council, Wake Forest University, 1981-84
Dean's Fellowship Committee, 1983-84
Chairman, Biosafety Committee, 1981-82
University Senate, Ad Hoc Committee, 1982-83
Chairman, Graduate Student Committee, 1980-85
Member, Undergraduate Education Subcommittee, 1984
Faculty Executive Council, 1983-85
Member, Search Committee for Department of Medicine Chairperson, 1985
Vice Chairman, Animal Care and Use Committee, 1985-1991
Associate Director, P-3 Facility, 1987-present
Longitudinal Evaluation Committee, 1984-1988
Committee to Select Faculty Teaching Excellence Award, 1987, 1990
Medical Student Promotions and Progress Committee, 1987-1990

Medical Student Promotions and Progress Committee, 1987-1990
Chairman, Animal Care and Use-Public Affairs Committee, 1988-1990
Chairman, Public Affairs Committee-Office of Research Development, 1990-1999
Evaluation of the Parallel Curriculum, 1992
Miniphase Parallel Curriculum Committee, 1990-1993
Graduate Program Director, Department of Microbiology and Immunology (1992-1994)
Wake Forest University Senators (1993-2001)
Chairman, Longitudinal Evaluation Committee, Wake Forest University Senate (1993-1997)
Chairman, Research! North Carolina Planning Committee Northwest AHEC Region, 1993
Member-Legal University Subcommittee of the Advisory Committee on Development and Transfer of Biotechnology 1993-1994.
Member Search Committee for Radiation Therapy Chairperson- 1994
Member Ad Hod Technology Transfer Subcommittee – 1994
Committee Chairperson, Pilot Projects Review Committee, Comprehensive Cancer Center of Wake Forest University (1995-1997)
Wake Forest University Senate Ad Hoc Committee (1997-1998)
United Way Campaign, Faculty Coordinator, Department of Microbiology and Immunology (1999).
Team Leader, Environmental Health and Safety, Department of Microbiology and Immunology – present
Animal Care and Use Committee-Alternate member - present
Heighten Awareness Committee - present
Public Affairs Committee (PACOR) – present
Executive Committee, Department of Microbiology and Immunology - present

## OTHER PROFESSIONAL APPOINTMENTS AND ACTIVITIES:

**Extramural:**
Editorial Board Member, Infection and Immunity, 1978-1980
Reviewer, Human Cell Biology Program, NSF, Ad Hoc
Contract Reviewer for the National Institute of Environmental Health Sciences Site Visit Reviewer, National Cancer Institute, Ad Hoc
Reviewer, Clinical Chemistry
Invited Reviewer, Proceedings of the National Academy of Sciences, Ad Hoc Reviewer Journal of General Virology
Grant Reviewer, North Carolina Biotechnology Program
Reviewer, Microbiology and Immunology Test Item Bank, Association of Medical School Microbiology Chairmen
Site Visit Staff Member, Reaffirmation Accreditation Team, Southern Association of Colleges and Schools, 1988
Scientific Reviewer for Montana University System, 1989
NIH, Study Section Member, AIDS and Related Review Group, Subcommittee 3, 1989
Scientific Reviewer for North Carolina University System, 1989
Ad Hoc Study Section member, Animal Resources Review Committee, NIH, 1991

Chairman, research!North Carolina Planning Committee, Northwest AHEC Region 1993
Board Member of the North Carolina Association for Biomedical Research, 1993-1999
Member of the North Carolina Steering Committee, Research!North Carolina, 1993
The Legal-University Subcommittee of the Advisory Committee on Development and Transfer of Biotechnology (1993-1994)
Executive Board Member, North Carolina Association for Biomedical Research (1993-1996)
Member, North Carolina Association for Biomedical Research (1993-2001)

**Sabbatical Leave:**
German Cancer Research Center, Institute for Virus Research, Heidelberg, West Germany, January-August, 1986.
Boehringer Mannheim Pharmaceutical Company, Mannheim, Germany (March-May, 1996)

## PROFESSIONAL INTERESTS:

### Current Research Interests:

<u>Development of new strategies for anti-HIV agents</u>

Statement of Research Objectives:

My major research objectives are to develop and evaluate innovative strategies for attacking the virus etiologic agent of AIDS with ether lipid (EL) analogs that are membrane interactive and have potent anti-HIV activity so that rationale predictions can be made on the clinical effectiveness of these novel entities as chemotherapeutic drugs for AIDS. These new entities have great clinical significance for designing new strategies for chemotherapy of AIDS because EL are membrane interactive and not DNA interactive and they are not associated with myelosuppression or neuropathology based on recent Phase I/Phase II trials in humans as anti-cancer drugs. Two classes of complex lipids, 1) synthetic phospholipids e.g alkylthioetherglycerophosphocholines and alkylamidopropylphosphocholines, and 2) phosphate ester-linked lipid-AZT (EL-AZT) conjugates, have demonstrated selective and potent activity against infectious virus replication and pathogenesis in human T-cells and promonocytic-like cells. Preliminary data indicated that selected ether lipids alone and EL-AZT conjugates are both active against AZT-resistant HIV-1, are bioavailable after oral administration, are not myelosuppressive, and they inhibit retrovirus infection and pathogenesis in animals. Based on these interesting results, PhaseI/II clinical trials with an optimal thioether lipid-nucleoside conjugate have been initiated in HIV+ volunteers by Boehringer Mannheim GmbH. The major objective of the proposed research is to enhance the principle of combination therapy to a molecular combination of two anti-HIV agents that will multitarget the HIV replication cycle. The proposed synthetic compounds will consist of a thio- or amido- phosphocholine lipid (PC lipid) conjugated with AZT (PC lipid-AZT). The hypothesis is that newly synthesized optimal double targeting PC lipid-nucleoside

conjugate should be more effective against HIV compared to our lead compounds INK-11 (phosphocholine lipid alone) and INK-14 (lipid-nucleoside conjugate where AZT is linked at position-3 of the lipid backbone through a phosphodiester bond in place of phosphocholine), accumulate to lyphoid tissues and cross the blood-brain barrier after oral administration. The Specific Aims are: (I) To synthesize and evaluate in vitro the cytotoxicity and anti-HIV-1 activity of novel thio- and amido-PC lipid and PC lipid-AZT conjugates. (II) To confirm and enhance in vitro and in-vivo the antiviral potency of selected compounds. (III) To characterize the mode of action of optimal compounds. (IV) To evaluate optimal compounds for capacity to accumulate into lymphoid tissues and cross the blood-brain barrier. The pharmacodynamic advantages and novel membrane site of action of PC lipid-AZT conjugates should provide new modalities for delivering combinations of drugs that will be orally bioavailable and target the virus at multiple sites in the replication cycle.

## GRANTS: PAST, CURRENT AND PENDING:

9/1/70 - 8/31/71 Forsyth Cancer Service
Winston-Salem, North Carolina
Investigation on Herpes-Type Viruses
and Tumorigenesis
$1,694.60

Fluid Research Fund
Bowman Gray School of Medicine
Sequential Protein Synthesis in Frog Polyhedral Cytoplasmic Deoxyribovirus Infected Mammalian Cells
$1,305.25

8/2/71 - 8/1/72` Forsyth Cancer Service
Investigations on Herpes-Type Viruses and Tumorigenesis
$2,200

10/1/71 - 9/30/74 National Cancer Institute, NIH
Virus Associated Neoplasms
(CA 12382-01 to 03)
$58,005

3/1/72 - 2/28/75 National Cancer Institute, NIH
Herpesviruses and the Etiology of the Lucke Renal Adenocarcinoma and Human Cervical Carcinoma
(CA 12197-01 to 03)
$105,000

10/1/74 - 11/30/77 National Cancer Institute, NIH

Virus Associated Neoplasms
(CA 12382-04 to 06)
$117,566

3/1/75 - 2/28/77 National Cancer Institute, NIH
Herpesviruses and Etiology of Human Carcinomas
(CA 12197-03 to 04)
$43,400

9/1/77 - 8/31/80 National Cancer Institute, NIH
Changes in Cell Membranes by Oncogenic
Herpes Simplex
$99,054
(M. Waite, Principal Investigator;
L. Kucera, Collaborator)

9/1/79 - 8/31/82 Environmental Protection Agency
Herpes Simplex Virus Type 2 -
Cancer Promotor Interactions
$157,896

Environmental Protection Agency
Prostaglandins as Markers of Promotor-Virus
Cell Transformation
$247,629
(M. Waite, Principal Investigator;
L. Kucera, Collaborator)

9/15/80 - 1/31/94 National Cancer Institute, NIH
Tissue Culture Core Laboratory
$49,514 (current year)
Provide expertise, equipment and service to principal investigators in the Oncology Research Center at Bowman Gray School of Medicine

11/1/80 - 10/31/84 Environmental Protection Agency
Prostaglandins as Markers of Promoter-Virus Cell Transformation
$103,535
(M. Waite, Principal Investigator; L. Daniel, Co-Investigator; L.S. Kucera, Collaborator)
Experiments are being done to determine the origin and structure of the prostan:ids produced upon exposure of transformed cells to tumor promoters (12-0-tetradecanoyl-phorbol-13-acetate).

6/1/81 - 5/31/83 National Cancer Institute, NIH CA 12197
Tumor Promoter Interaction with Viral DNA in Herpes Simplex Virus Type 2 Transformed Cells
$4,700.00

(Kilpatrick, Principal Investigator;
L.S. Kucera, Co-Investigator)

9/1/81 - 8/31/83 Forsyth Cancer Service
An Experimental Model for Elucidating a Possible Multistep Process in Human Carcinogenesis
$1,500

9/15/82 - 9/14/84 Pilot Grant Funds
Department of Radiology
Development of Monoclonal Antibodies to a Tumor-Associated Antigen Ag4
$11,800
(L.S. Kucera, Principal Investigator;
M.J. Ricardo, Co-Investigator)
Purified Ag4 generated in our laboratory is being used to develop monospecific antibodies. The monospecific antiserum will be used to localize primary and metastatic tumors and to target toxic agents to herpes simplex virus type 2 transformed cells.

7/1/83 - 6/30/84 Intramural Research Support
Bowman Gray School of Medicine
The Consequences of Ovarian Hormones-Herpes Simplex Virus Type 2 Interactions on the Phenotypic Properties of the Uterine Cervix of C3H Mice
$4,295
(W.J. Bo, Principal Investigator; L.S. Kucera, Co-Investigator)

9/15/83 - 9/14/85 Forsyth Cancer Service
Consequences of Phorbol Diester Stimulation of Prostaglandin Synthesis and Cocarcinogen Metabolism During Herpes Simplex Virus Carcinogenesis
$3,000
(Catherine Krebs, Student Investigator; Louis S. Kucera, Sponsor)

10/1/83 - 9/30/87 National Heart and Lung Institute, NIH
Functional Development of Neonatal Alveolar Macrophages
$368,119
(Q.N. Myrvik, Principal Investigator; L.S. Kucera, Co-Investigator)

11/1/86 - 10/31/88 DuPont, Inc.
Development of Herpesvirus-induced Ag4/ICP10 Tumor Antigen for use in the Immunoprognosis of Human Cervical Carcinoma
$20,500
(L.S. Kucera, Principal Investigator)

12/1/87 - 11/30/88 NIH
Investigation of Retrovirus Prevalence in the Nonhuman Primate Colony at BGSM
$9,550
(L.S. Kucera, Principal Investigator)

1/1/88 - 12/31/88 R.J. Reynolds Tobacco Co.
Macrophage Migration Enhancement Factor and Natural Suppressor Cells
$108,864
(Q.N. Myrvik, Principal Investigator; L.S. Kucera, Co-Investigator)

4/1/88 - 3/31/91 National Heart and Lung Institute, NIH
Functional Development of Neonatal Alveolar Macrophages
$488,647
(Q.N. Myrvik, Principal Investigator; L.S. Kucera, Co-Investigator)

9/1/88 - 8/31/90 Forsyth Cancer Service
Investigations of a Novel Bacterial Cytolysin with Potent Killing Activity of Human Leukemia Cells
$4,000
(L.S. Kucera, Principal Investigator; A.S. Kreger, Co-Investigator)

6/1/90 - 5/31/91 Boehringer Mannheim GmbH
Evaluation of Ether Lipid Compounds for Anti-HIV Activity
$112,540
(L.S. Kucera, Principal Investigator; C. Piantadosi, Co-Investigator)

9/1/89 - 11/30/92 NIH
NCDDG for the Treatment of AIDS: Nucleosides and Thiosemicarbazones as Selective Inhibitors of HIV
$147,419
(L.S. Kucera, Program Leader for Program II)

9/7/92 - 3/6/93North Carolina Biotechnology Center
Events Support Grants Program Animals, Vaccines, Polio and AIDS
$2,500
(L.S. Kucera, Principal Investigator)

1/1/93 - 12/31/94 Sphinx Pharmaceuticals Corporation
In-Vitro Evaluation of Novel Ether Lipid Analogs for Cell Cytotoxicity and Activity Against HIV and Hepatitis B Virus Infection
$240,000
(L.S. Kucera, Principal Investigator)

10/27/93 - 12/31/93 North Carolina Biotechnology Center
Educational Meetings Grants Program From Molecules to the Mind; The Use of Animals in Biomedical Research
$2,375
(L.S. Kucera, Principal Investigator)

6/1/94 - 8/15/94 Pediatric Aids Foundation

Student Intern Award
$2,000
L.S. Kucera, Principal Investigator

12/15/95 – 12/14/97 Boehringer Mannheim GmbH
Biological Evaluations of Optimal Anti-HIV-1 Synthetic Phospholipid-Conjugates
$200,000.
(L. S. Kucera, Principal Investigator)

02/01/97 – 01/31/98 NIH-NCI
Tissue Culture Core Laboratory
$62,425
(L. S. Kucera, Core Laboratory Director)

02/01/98 – 01/31/00
Tissue Culture Core Laboratory
$131,664
(L.S. Kucera, Core Laboratory Director)

04/01/97 – 03/31/02 NIH-NIAID
Interaction of HIV, Macrophages and Salmonella
$1,042,898
(S. B. Mizel, Principal Investigator; L. S. Kucera, Co-Investigator)

10/01/99 – 09/29/01 NIH-NIAID
Simian Virus 5 as an HIV Vaccine Vector
$200,000
(G. Parks, Principal Investigator, L.S. Kucera, Co-Investigator)

12/15/00 – 02/28/02 Eli Lilly & Co.
Use of Synthetic Phospholipids for Drug Delivery
$134,195
L.S. Kucera, Principal Investigator

01/30/02 – 01/29/03 Kucera Pharmaceutical Company
Pharmacologic Activity of Novel Synthetic Phospholipid-AZT Conjugates in HIV Infected Cells
$136,030
L.S. Kucera, Principal Investigator

02/01/01 – 01/31/07 NIH-NCI
Cell Culture and Virus Vector Core Laboratory
$382,027
(L.S. Kucera, Core Laboratory Director)

**PATENTS:**

Piantadosi C, Ishaq KS, Marasco C Jr, Daniel LW, Kucera LS, Modest EJ, Goz BP. U.S. Patent Application Serial No. 07/262, 458, Filed 25 October 89 for Quaternary Ammonium Derivatives of Alkylglycerols. Patent Number: 5,614,548

Piantadosi C, Kucera LS, Marasco C Jr, Meyer KL. A Family of Phosphorus-Containing Lipid Nucleoside Analogs as Anti-HIV-1 Agents. U.S. Patent Application Serial No. 07/539, 001, Filed 15 June 91 for Ether Lipid-Nucleoside Conjugates. Patent Number: 5,512,671

Townsend LB, Drach JC, Kucera LS. U.S. Patient Application Serial No. 07/687, 579, Filed 19 April 91 for Triciribine and Analogs as Antiviral Drugs. Patent Number: 5,633,235

Kucera LS, Morris-Natschke S. U.S. Patent Application, Filed 10 June 93 for Method of Combatting Hepatitis B Virus Infection.

Kucera LS, Morris-Natschke SL, Ishaq KS. U.S. Patent Application, Filed 29 August 94 for Method of Combatting Viral Infections and Compounds for Same.

Kucera LS, Fleming RA, Ishaq KS, Kucera GL, Morris-Natschke SL. U.S. Patent filed April 27, 2001, 09/844,201 Compositions and Methods for Double-Targeting Virus Infections and Targeting Cancer Cells.

**PROFESSIONAL MEMBERSHIPS:**

Sigma Xi
American Society for Microbiology
American Association for the Advancement of Science
International Agency for Research on Cancer
Fellow in the American Academy of Microbiology
American Society for Virology
New York Academy of Sciences
International AIDS Society
International Society for Antiviral Research

**DIRECTORY LISTINGS:**

Who's Who in the South and Southeast
American Men and Women of Science
North Carolina Scientists in Biotechnology

**COMMUNITY ACTIVITIES:**

Member, Diocese of Charlotte Board of Education
Representative, United Fund Campaign
Representative, YMCA Building Fund
Executive Board Member, Winston-Salem Area Soapbox Derby, Inc.
Treasurer, Parent Teachers Association, Our Lady of Mercy School
President, Parent Teachers Association, Our Lady of Mercy School
Chairman, Education Commission, Our Lady of Mercy Church
Parish Council, Our Lady of Mercy Church

**PERSONAL INTERESTS:**

Golf, Camping, Hiking, Sportscar Buff, Foreign Travel

**LECTURES, SPEECHES, EXHIBITS, FILMS:**

Kucera LS. Oncogenic properties of a continuous line of Lucke tumor cells. In Spear P, Nahmias A. Abstracts of papers presented at the meeting on the herpes viruses, August 17-20, 1973. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1973.

Kucera LS, Gusdon JP. Oncogenic transformation of rat embryo fibroblasts with photoinactivated herpes simplex virus type 2. In: Pagano JS, Shubak-Sharpe J, eds. Abstracts of papers presented at the third meeting on herpes viruses, August 31 - September 4, 1976. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1976.

Kucera LS, Edwards I. Stable properties of clonal lines of rat embryo cells transformed with photoinactivated herpes simplex viruses. Harvard University, Cambridge, Massachusetts, 1977.

Kucera LS, Edwards I. Herpes simplex virus type 2 (HSV-2) functions expressed during stimulation of human cell DNA synthesis. Herpes Virus Workshop. Program and abstracts, St. John's College, Cambridge, England 1978.

Kucera LS, Hale AH, Daniel LW, Waite BM. Parameters distinguishing herpes simplex virus type 2 transformed tumorigenic and nontumorigenic rat cells. In: Kieff E, Schaffer, eds. Abstracts of papers presented at the Fourth Cold Spring Harbor Meeting on Herpesviruses, August 28 - September 2, 1979. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1979.

Kucera LS, Edwards I, Respess RA. The tumor promoter (tetradecanoyl-phorbol-13-acetate) enhances the malignant potential of herpes simplex virus, type 2 transformed cells. In: Hayward GS, Summers WC, eds. Abstracts of papers presented at the Fifth Cold Spring Harbor Meeting on Herpesviruses. Cold Spring Harbor, NY: Cold Spring Laboratory 1980.

Respess RA, Kucera LS, Waite BM. Aminoacyl-fucosides as in vitro markers of tumorigenic potential of tetradecanoyl phorbol acetate (TPA) treated and untreated herpes simplex virus, type 2, (HSV-2) transformed rat cells. In: Hayward GS, Summers WC, eds. Abstracts of papers presented at the Fifth Cold Spring Harbor Meeting on Herpesviruses. Cold Spring Harbor, NY: Cold Spring Laboratory, 1980.

Lewis J, Kucera LS, Eberle R, Courtney R. Identification of specific herpes simplex virus type 2 (HSV-2) glycoproteins expressed in HSV-2 transformed cells. In: Hayward GS, Summers WC, eds. Abstracts of papers presented at the Fifth Cold Spring Harbor Meeting on Herpesviruses. Cold Spring Harbor, NY: Cold Spring Laboratory, 1980.

Raben M, Heise ER, Kucera LS, Witcofski R, Plunkett S. Effects of radiation on lymphocyte populations. Int Symp in Immunoplasmic Effects of Radiation Therapy, Montpellier, France, March 13-15, 1980.

Kucera LS. Herpes simplex virus type 2 - tumor promotor interaction: a model for two-stage carcinogenesis. In: Kaplan AS, La Placa M, Rapp F, Roizman B, eds. International Workshop on Herpesviruses. Bologna, Italy: Societa Editrice Esculapio 1981.

Kucera LS, Respess R, Daniel LW, Waite BM. Metabolic consequences of cocarcinogen-HSV-2 transformed cell interaction. American Society for Virology, Cornell University, August 2-5, 1982.

Kucera LS, Respess R, Daniel LW, Waite BM. Phorbol diester (TPA) promotion of carcinogenesis of cells initiated in transformation by herpes simplex virus type 2 (HSV-2). 13th International Cancer Congress, Seattle, Washington, September 8-15, 1982.

Flanders RT, Kucera LS, Ricardo MJ Jr, Plunckett S, Raben M. Characterization of an HSV-2 tumor-associated antigen Ag4 reactive with cervical carcinoma patients' sera. American Society for Virology, Michigan State University, July 10-14, 1983.

Krebs CR, Kucera LS, Waite BM. Potential cofactor role of prostaglandin synthesis in herpes simplex virus type 2 (HSV-2) carcinogenesis. American Society for Virology, Michigan State University, July 10-14, 1983.

Kucera LS, Krebs C, Waite BM. Herpes simplex virus transformed cell-cocarcinogen interactions: possible role of prostaglandin synthesis in promotion of carcinogenesis. 8th International Herpesvirus Workshop, Oxford, England, July 31 - August 5, 1983.

Flanders RT, Kucera LS, Raben M, Ricardo MJ. Immunochemical characterization of herpes simplex virus type 2 (HSV-2) antigens ICPIO and ICSP 11/12. 9th International Herpesvirus Workshop, University of Washington, August 24-29, 1984.

Krebs C, Kucera L, Waite M. Stimulation of prostaglandin synthesis in herpes simplex

virus type 2 (HSV-2) transformed cells by combinations of phorbol diesters and chemical carcinogens. 9th International Herpesvirus Workshop, University of Washington, August 24-29, 1984.

Kucera LS, Willcox M, Ricardo MJ Jr, Myrvik QN. Age - dependent functions of rabbit alveolar macrophages: resistance to herpes simplex virus type 2 infection and interferon production. 10th International Herpesvirus Workshop, University of Michigan, Ann Arbor, MI, August 11-16, 1985.:

Flanders RT, Iyer N, Brokaw J, Ricardo M Jr, Kucera LS. Reactivity of rabbit polyclonal Ag4/ICp10 antibodies with HSV-2 induced ribonucleotide reductase and human cervical carcinoma cells. 10th International Herpesvirus Workshop, University of Michigan, Ann Arbor, MI, August 11-16, 1985.

Krebs CR, Jariwalla R, Aurelian L, Waite M, Kucera LS. Functions induced by the tumorigenic region (Bgl II C) of herpes simplex virus. 11th International Herpesvirus Workshop, The University of Leeds, England, July 21-26, 1986.

Krebs CR, Kucera LS, Jariwalla R, Waite M. The oncogenic region of herpes simplex virus type 2 (HSV-2) DNA induces the capacity for 12-0-tetradecanoylphorbol-13-acetate stimulation of prostaglandin synthesis in transformed rat cells. The American Society for Virology, 1986 Annual Meeting, University of California, Santa Barbara, CA, June 22-26, 1986.

Krebs CR, Waite M, Kucera LS. A cell-free assay for prostaglandin synthetase provides new evidence that the enzyme is transiently induced during herpes simplex virus type 2 cell transformation. The American Society for Virology, 1987 Annual Meeting, University of North Carolina, Chapel Hill, NC, May 31-June 4, 1987.

Sieling PA, Waite M, Kucera LS. Arachidonate metabolism and protein kinase C with activity alteration in cells cotransfected with an antibiotic resistent plasmid (pSV2 neo) and herpes simplex virus type 2 DNAS. 13th International Herpesvirus Workshop, University of California, Irvine, August 7-13, 1988.

Kucera L, Raben D, Iyer N, Ricardo M Jr, Leake E, Heise E, Myrvik Q. Evidence that some transformed human T-cells can be coinfected with herpes simplex virus type 2 (HSV-2) and human immunodeficiency virus (HIV). 13th International Herpesvirus Workshop, University of California, Irvine, August 7-13, 1988.

Kucera LS, Leake E, Raben D, Iyer N, Myrvik Q. Electron microscopic evidence for coinfection and replication of herpesvirus and HIV-1 in the same human T-cell. 5th International Conference on AIDS, Montreal, Quebec, Canada, June 4-9, 1989.

Kucera LS, Iyer N, Raben A, Modest E, Daniel L, Piantadosi C. Inhibition of HIV-1 plaque formation by a novel class of membrane-active ether lipid analogs. 5th International Conference on AIDS, Montreal, Quebec, Canada, June 4-9, 1989.

Meyer KL, Morris-Natschke S, Marasco CJ, Ishaq KS, Piantadosi C, Kucera LS. Synthesis and evaluation of anti-HIV-1 ether lipids. AAPS, Atlanta, Georgia, October 22-25, 1989, p.S-41.

Kucera LS, Iyer N, Leake E, Raben A, Daniel L, Modest E, Piantadosi C. Effect of membrane active ether lipid (EL) analogs on human immunodeficiency virus production measured by plaque assay. 2nd International Conference on Drug Research In Immunologic and Infectious Diseases, AIDS, Arlington, Virginia, November 6-9, 1989, Session II-1.

Modest EJ, Noseda A, Wallen CA, Kucera LS, Piantadosi C. Combination chemotherapy studies with antitumor and antiviral ether lipid analogs. 81st Annual Meeting of the American Association for Cancer Research. Washington, DC, May 23-26, 1990.

Marasco C, Piantadosi C, Meyer K, Kucera L. The synthesis, biological evaluation, and structure activity of amido phosphocholines and related analogs as anti-HIV-1 agents. 6th International Conference on AIDS. San Francisco, CA, June 20-24, 1990.

Giridhar G, Hayakawa H, Kucera LS, Myrvik QN. Effect of herpes simplex virus type 2 infection on the respiratory response in infant and adult rabbit alveolar macrophages. 15th International Herpesvirus Workshop. Georgetown University, Washington, DC, August 2-8, 1990.

Modest EJ, Noseda A, Daniel LW, Wallen CA, Kucera LS, Piantadosi C. Antineoplastic and antiviral properties of ether lipid analogs. 15th International Cancer Congress. Hamburg, Germany, August 16-22, 1990.

Krugner-Higby L, Iyer N, Kucera L. Granulocyte macrophage-colony stimulating factor stimulates persistently infected U1 cells to produce infectious human immunodeficiency virus (HIV). 8th Annual Symposium on Nonhuman Primate Models for AIDS. New Orleans, LA, November 28-30, 1990.

Pudlo JS, Drach JC, Kucera LS, Kahlon JB, Shannon WM, Townsend LB. Synthesis of branched cytidine and uridine analogs and activities against human immunodeficiency virus and herpes viruses. 30th Annual International Conference on Antimicrobial Agents and Chemotherapy. Atlanta, GA, October 21-24, 1990.

Kucera LS, Iyer N, Leake E, Raben A, Daniel L, Modest E, Piantadosi C. Inhibition of human immunodeficiency virus-1 (HIV-1) by novel membrane interactive ether lipids. 81st Annual Meeting of the American Association for Cancer Research. Washington, DC, May 23-26, 1990.

Kucera LS, Iyer N, Puckett S, Meyer K, Morris-Natschke S, Marasco C, Piantadosi C, Modest E. Investigations on the mechanism of action of novel anti-HIV ether lipid

analog. 25th Annual Southeastern Regional Lipid Conference, High Hampton Inn, Cashiers, NC, October 24-26, 1990.

Kucera LS, Iyer N, Puckett S, Buckheit RW, Hollingshead M, Westbrook L, Toyer B, White EL, Germany J, Shannon WM. Activity of tricyclic nucleoside and tricyclic nucleoside 5' phosphate (triciribine) against HIV and other retroviruses. 4th International Conference on Antiviral Research, New Orleans, LA, April 21-24, 1991.

Kucera LS. Novel anti-HIV ether lipids: investigations on structure-activity relationship and mode of action. The University of Michigan, School of Dentistry, Department of Biologic and Materials Sciences and College of Pharmacy, Department of Medicinal Chemistry, Ann Arbor, MI, February 27, 1991.

Drach JC, Townsend LB, Shipman C, Jr, Wotring LL, Nassiri MR, Kucera LS, Buckheit RW, Shannon WM. Activity of triciribine and triciribine monophosphate against human immunodeficiency virus and cytotoxicity in human cells. Fourth Conference of the NIH National Cooperative Drug Discovery Groups for the Treatment of HIV Infection, San Diego, CA, November 3-7, 1991.

Kucera LS. In-vitro evaluation of ether lipid and nucleoside analog combinations against infectious HIV-1 production. Combination Therapies in AIDS: Issues and Perspectives. Food and Drug Administration and the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, November 19-21, 1991.

Giridhar G, Myrvik QN, Kucera LS. TPA induced replication of infectious HSV-2 replication in infant and adult rabbit alveolar macrophages. The 1992 Annual Meeting, The American Society for Virology, Cornell University, Ithaca, NY July 11-15, 1992.

Kucera LS. Mechanisms of action of novel membrane active anti-HIV-1 ether lipids. East Carolina University School of Medicine, Greenville, NC September 1, 1992.

Kucera LS, Krugner-Higby LA, Iyer NP, Goff DH, Edwards TV, Neufeld JA, Anand IS, Puckett S, Morris-Natschke SL, Piantadosi C. Investigations on membrane active ether lipid analogs that alter functional expression of HIV-1 induced glycoproteins and inhibit pathogenesis. A Wellcome Symposium In Antiviral Therapy: Innovations in Therapy of Human Viral Diseases. Sheraton Imperial Hotel and Convention Center, Research Triangle Park, NC December 6-9, 1992.

Kucera LS, Iyer N. Wake Forest University Medical Center, Winston-Salem, NC, 27157 U.S.A. and Morris-Natschke S, Piantadosi C. University of North Carolina, Chapel Hill, NC, 27599 U.S.A. Novel membrane active ether lipids that inhibit HIV-1 induced gp160/120 functional expression and pathogenesis. IXth International Conference on AIDS in affiliation with the IVth STD World Congress, Berlin, Germany, June 6-11, 1993.

Kucera LS, Iyer N. Wake Forest University Medical Center, Winston-Salem, NC, 27157

U.S.A., Morris-Natschke S, University of North Carolina, Chapel Hill, NC 27599 U.S.A. and Marasco C, Roswell Park Cancer Institute, Buffalo NY 14263. Combinations of ether lipid analogs, AZT, and oligodeoxynucleotides against AZT-resistant and wild-type HIV-1 replication. 5Th International Conference of the NCDDG-HIV. Coordinated Therapies for HIV Infection. Georgetown University Conference Center, Washington, DC July 11-16, 1993.

**BIBLIOGRAPHY:**

**Monographs and Textbooks:**

Acton JD, Kucera LS, Myrvik QN, Weiser RS. Fundamentals of medical virology for students of medicine and related sciences. Philadelphia: Lea & Febiger, 1974.

Kucera LS, Myrvik QN. Fundamentals of medical virology, 2nd ed. Philadelphia: Lea & Febiger, 1985.

**Chapters in Books:**

Kucera LS, Herrmann EC Jr. Antiviral agents. In: Cain CK, ed. Annual report in medicinal chemistry. New York: Academic Press, 1966:129-262.

Raben M, Heise ER, Kucera LS, Hale AH, Witcofski RL, Plunkett S. Effects of irradiation on lymphocyte subpopulations. In: Dubois JB, Serron B, Rosenfield C, eds. Immunopharmacologic effects of radiation therapy. New York: Raven Press, 1981:321-328.

Kucera LS. Immunity to viruses. In: Myrvik QN, Weiser RS, eds. Fundamentals of immunology. Philadelphia: Lea and Febiger, 1983:425-443.

Kucera LS. Morphology growth and genetics of animal viruses. In: Rose NR, Barron AL, eds. Microbiology: basic principles and clinical applications. New York: Macmillan, 1983.

Kucera LS, Piantadosi C, Modest EJ. Novel membrane interactive ether lipids with anti-HIV activity. In: Aloia RC, Curtain CC, Gordon LM, eds. Membrane interactions of human immunodeficiency virus. Advances in Membrane Fluidity Series, Volume 6. New York: John Wiley & Sons, Inc., 1992:pp329-350.

Ishaq, K., Morris-Natschke, S., Iyer, N., Kucera, L.. Acyclic nucleosides containing boronic acid target HIV-1. In: Monduzzi Editore. 12th World AIDS Conference, Geneva (Switzerland), June 28-July 3, 1998, Clinical Science Vol 2. Bologna, Italy: Monduzzi Editore, 1998:485-488..

Doellgast GJ, Beard GA, Sheehan M, Iyer N, Kucera LS, Richardson SH. Amplified Assay for Specific Dual-Labelled DNA Using the Coagulation Cascade (EDNA-ELCA)..

In: Bernd Kochanowski and Udo Reischl. Methods in Molecular Medicine: Quantitative PCR Protocols. : Humana Press Inc., 1999:197-21

## PEER REVIEWED PUBLICATIONS:

Kucera LS, Goldberg HS, Goodman RN. Conversion of penicillin resistance to penicillin susceptibility in staphylococcus aureus. Antimicrob Agents Chemother 1963;489-492.

Cohen RA, Kucera LS, Herrmann EC Jr. Antiviral activity of Melissa officinalis (lemon balm) extract. Proc Soc Exp Biol Med 1964;117:431-434.

Kucera LS, Goldberg HS, Goodman RN. Influence of 2,4,5-trichlorophenoxyacetic acid on Staphylococcal penicillinase. J Bacteriol 1965;89:1621-1622.

Kucera LS, Cohen RA, Herrmann EC Jr. Antiviral activities of extracts of the lemon balm plant. Ann NY Acad Sci 1965;130:474-482.

Kucera LS, Herrmann EC Jr. Gradient plate technique applied to the study of antiviral substances. Proc Soc Exp Biol Med 1966;122:258-262.

Kucera LS, Herrmann EC Jr. Antiviral substances in plants of the mint family (Labiatae). I. Tannin of Melissa officinalis. Proc Soc Exp Biol Med 1967;124:865-869.

Herrmann EC Jr, Kucera LS. Antiviral substances in plants of the mint family (Labiatae). II. Nontannin polyphenol of Melissa officinalis. Proc Soc Exp Biol Med 1967;124:869-874.

Herrmann EC Jr, Kucera LS. Antiviral substances in plants of the mint family (Labiatae). III. Peppermint (Mentha piperita) and other mint plants. Proc Soc Exp Biol Med 1967;124:874-878.

Kucera LS, Granoff A. Viruses and renal carcinoma of Rana pipiens. VI. Interrelationships of macromolecular synthesis and infectious virus production in frog virus 3-infected BHK 21/13 cells. Virology 1968;34:240-249.

McAuslan BR, Kucera LS. DNA polymerase activity in FV3-infected BHK cells. Virology 1968;35:328-330.

Kucera LS, Granoff A. Induction and regulation of DNA nucleotidyltransferase activity in fish cells infected with frog virus 3. Virology 1969;37:455-463.

Kucera LS. Effects of temperature on frog polyhedral cytoplasmic deoxyribovirus multiplication: thermosensitivity of initiation, replication, and encapsidation of viral DNA. Virology 1970;42:576-589.

Kucera LS. Failure of rifampin to inhibit frog polyhedral cytoplasmic deoxyribovirus multiplication. Antimicrob Agents Chemother 1973;4:372-375.

Tara CS, Stanley JA, Kucera LS, Hollis S. Photodynamic inactivation of herpes simplex keratitis. Arch Ophthalmol 1974;92:51-53.

Kucera LS, Simonson J. Isolation and characterization of a cell line from the cocultivation of Lucke renal tumor cells and nontransformed feeder cells. J Natl Cancer Inst 1974;53:415-421.

Gaby NS, Kucera LS. DNA-dependent RNA polymerase activity associated with subviral particles of polyhedral cytoplasmic deoxyribovirus. J Virol 1974;14:231-238.

Gusdon JP Jr, Kucera LS. The oncogenic potential of dye-light treatment of herpetic lesions. Am J Obstet Gynecol 1974;120:569-570.

Melvin P, Kucera LS. Induction of human cell DNA synthesis by herpes simplex virus type 2. J Virol 1975;15:534-539.

Kucera LS, Gusdon JP. Transformation of human embryonic fibroblasts by photodynamically inactivated herpes simplex virus, type 2 at supra-optimal temperature. J Gen Virol 1976;30:257-261.

Marcon MJ, Kucera LS. Consequences of herpes simplex virus type 2 and human cell interaction at supra-optimal temperatures. J Virol 1976;10:54-62.

Kucera LS, Gusdon JP, Edwards I, Herbst G. Oncogenic transformation of rat embryo fibroblasts with photoinactivated herpes simplex virus: rapid in vitro cloning of transformed cells. J Gen Virol 1977;35:473-485.

Kucera LS, Leake ES, Edwards I, Wright MJ. Biologic and morphologic properties of a new ascites cell line derived from a Lucke renal adenocarcinoma-bearing Rana pipiens. J Reticuloendothel Soc 1977;22:349-362.

Waite BM, Kucera LS, King L, Crosland S. Lipid synthesis in cultured human embryonic fibroblasts. Lipids 1977;12:698-706.

Kucera LS, Edwards I. Herpes simplex virus type 2 functions expressed during stimulation of human cell DNA synthesis. J Virol 1979; 29:83-90.

Kucera LS. Herpes simplex virus-host cell interactions. CRC Crit Rev Microbiol 1979;7:215-244.

Marcon MJ, Kucera LS. Stimulation of human cellular thymidine kinase activity during abortive infection with herpes simplex virus type 2. Virology 1979;96:315-318.

Marcon MJ, Kucera LS. Stimulation of human cell DNA synthesis by defective herpes simplex virus type 2. Virology 1979;98:364-372.

Heise ER, Kucera LS, Raben M, Homesley HD. Serological response patterns to herpes virus type 2 early and late antigens in cervical carcinoma patients. Cancer Res 1979;39:4022-4026.

Daniel LW, Kucera LS, Waite BM. Metabolism of fatty acids by cultured tumor cells and their diploid precursor fibroblasts. J Biol Chem 1980;255;5697-5702.

Hale AH, Kucera LS, Daniel L, Waite M. Parameters distinguishing herpes simplex virus type 2-transformed tumorigenic and nontumorigenic rat cells. Cancer Res 1981;41:629-634.

Respess RA, Edwards I, Kucera LS, Waite BM. Aminoacyl fucosides as possible biochemical markers of tumorigenic and metastatic potential in herpes simplex virus type 2-transformed rat cells. Cancer Res 1981;41:3507-3511.

Daniel LW, Waite BM, Kucera LS, King L, Edwards I. Phospholipid synthesis in human embryo fibroblasts infected with herpes simplex virus type 2. Lipids 1981;16:655-662.

Lewis J, Kucera LS, Eberle R, Courtney RJ. Detection of herpes simplex virus type 2 glycoproteins expressed in virus-transformed rat cells. J Virol 1982;43:275-282.

Kucera LS, Furman PA, Elion GB. Inhibition by acyclovir of herpes simplex virus type 2 morphologically transformed cell growth in tissue culture and tumor bearing animals. J Med Virol 1983;12:119-127.

Kucera LS, Daniel LW, Waite M. 12-0-Tetradecanoyl-13-Acetate (TPA) enhancement of the tumorigenic potential of herpes simplex virus type 2 transformed cells. Oncology 1983;40:357-362.

Kucera LS, Iyer N, King L, Krebs C, Waite BM. Calcium ionophore A-23187 and 12-0-tetradecanoyl-phorbal-13-acetate stimulation of prostaglandin synthesis in herpes simplex virus type 2 transformed rat cells. Cancer Res 1984;44:1625-1629.

Flanders RT, Kucera LS, Raben D, Ricardo MJ Jr. Immunologic characterization of herpes simplex virus type 2 antigens ICPIO and ICSP 11/12. Virus Res 1985;2:245-260.

Ricardo MJ Jr, Small WA, Myrvik QN, Kucera LS. Lipid composition of alveolar macrophage plasma membrane during postnatal development. J Immunol 1986;136:1054-1060.

Krebs CR, Waite M, Jariwalla R, Kucera LS. Induction of cellular functions in spontaneously immortalized rat-2 cells transfected with cloned herpes simplex virus type

2 (HSV-2) DNA. Carcinogenesis 1987;8:183-185.

Takata I, Chida K, Gordon MR, Myrvik QN, Kucera LS, Ricardo MJ Jr. L-fucose, L-mannose and L-galactose and their BSA conjugates stimulate macrophage migration. J Leukocyte Biol 1987;41:248-256.

Roddick VL, Krebs CR, Kucera LS, Daniel LW, Waite BM. Phospholipid-sensitive Ca2+-dependent protein kinase activity in herpes simplex virus type 2 transformed rat embryo fibroblasts. Oncology 1988;45:197-201.

Kucera LS, Raben D, Iyer N, Ricardo MJ Jr. Herpes simplex virus type 2 infection of unstimulated human T-lymphocytes. Viral Immunology 1989;2:11-16.

Krebs CR, Waite M, Kucera LS. Evidence for phospholipase and cyclooxygenase activities in herpes simplex virus type 2 transformed rat cells. Carcinogenesis 1989;10:2059-2065.

Krugner-Higby L, Kucera L, Lerche N, Sever J, Fucillo W, Allan J, Benveniste R. Serological survey for two simian retroviruses in macaques and African green monkeys. Lab Animal Sci 1990;40:24-28.

Kucera LS, Iyer N, Leake E, Raben A, Modest EJ, Daniel LW, Piantadosi C. Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation. AIDS Res Hum Retroviruses 1990;6:491-501.

Kucera LS, Leake E, Iyer N, Raben D, Myrvik QN. Human immunodeficiency virus type 1 (HIV-1) and herpes simplex virus type 2 (HSV-2) can coinfect and simultaneously replicate in the same human CD4+ cell: effect of coinfection on infectious HSV-2 and HIV-1 replication. AIDS Res Hum Retroviruses 1990;6:641-647.

Kucera LS, Iyer N, Leake E, Raben A, Daniel L, Modest E, Piantadosi C. Effect of membrane active ether lipid (EL) analogs on human immunodeficiency virus production measured by plaque assay. Proceed New York Acad Sci 1990;616:545-548.

Meyer KL, Marasco CJ, Jr., Morris-Natschke SL, Ishaq KS, Piantadosi C, Kucera LS. In-Vitro evaluation of phosphocholine and quaternary ammonium containing lipids as novel anti-HIV agents. J Med Chem 1991;34:1377-1383.

Piantadosi C, Marasco CJ, Jr., Morris-Natschke SL, Meyer KL, Gumus F, Surles JR, Ishaq KS, Kucera LS, Iyer N, Wallen CA, Piantadosi S, Modest EJ. Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV-1 activity. J Med Chem 1991;34:1408-1414.

Giridhar G, Hayakawa H, Kucera LS, Myrvik QN. Priming of rabbit alveolar macrophages for enhanced oxidative responses by herpes simplex virus type 2 infection. J Leukocyte Biol 1991;49:442-448.

Kucera L, Iyer N, Puckett S, Buckheit M, Hollingshead L, Westbrook B, Toyer EL, White J, Shannon W, Chen RC-S, Drach JC, Townsend LB. Activity of tricyclic nucleoside and tricyclic nucleoside 5' phosphate (triciribine) against HIV and other retroviruses. Antiviral Res 1991;Suppl. 1:48.

Hayakawa H, Giridhar G, Myrvik QN, Kucera L. Pulmonary surfactant phospholipids modulate priming of rabbit alveolar macrophages for oxidative responses. J Leuko Biol 1992;51:379-385.

Hsu L-Y, Wise DS, Kucera LS, Drach JC, Townsend LB. Synthesis of anti restricted pyrimidine acylic nucleosides. J Org Chem 1992;57:3354-3358.

Kucera LS, Iyer NP, Puckett SH, Buckheit RW, Jr., Westbrook L., Toyer BR, White EL, Germany-Decker JM, Shannon WM, Chen RC-S, Nassiri MR, Shipman C, Jr., Townsend LB, Drach JC. Activity of triciribine and triciribine-5'-monophosphate against human immunodeficiency virus types 1 and 2. AIDS Res Human Retroviruses 1993;9:307-314.

Swayze EE, Peiris SM, Kucera LS, White EL, Wise DS, Drach JC, Townsend LB. Synthesis of 1-(2-aminopropyl) benzimidazoles, structurally related to the TIBO derivative R82150 with activity against human immunodeficiency virus. Bioorganic Medicinal Chemistry Lett 1993;3:543-546.

Kasnar B, Wise DS, Kucera LS, Drach JC, Townsend LB. Synthesis of 2',3'- dideoxy- and 3' azido - 2',3' - dideoxy - pyridazine nucleosides as potential antiviral agents. Nucleosides and nucleotides, 1994;13:459-479.

Morris-Natschke, S.L., McLean, J.W., Ishaq, K.S., Goz, B., Iyer, N., Kucera, L.S. Novel lipid analogues as potential anti-HIV and antitumor agents. Pharmaceutical Research 1994. 11:S-120.

Kucera, L., Thompson, J., Iyer, N., Poduval, R., Morris-Natschke, S., Ishaq, K. Inhibition of human immunodeficiency virus envelope glycoprotein-mediated cell fusion by synthetic phospholipid analogs. 1995. Antiviral Res. 26: A260.

Mizel, S.B., Kucera, L.S., Richardson, S.H., Ciacci, F., and Iyer, N.P. Regulation of macrophage activation and HIV production by invasive Salmonella. Infection and Immunity, 63:1820-1826, 1995.

Krugner-Higby, L., Goff, D., Edwards,T., Iyer, N., Neufeld, J., Kute, T., Morris-Natschke, S., Ishaq, K., Piantadosi, C., and Kucera, L.S. Membrane interactive phospholipids inhibit HIV-1 induced cell fusion and surface gp160/gp120 binding to monoclonal antibody. AIDS Res. Human Retroviruses 11:705-712, 1995.

Krawczyk, S.H., Nassiri, M.R., Kucera, L.S., Kern, E.R., Ptak, R.G., Wotring, L.L., Drach, J.C., and Townsend, L.B. Synthesis and antiproliferative and antiviral activity of

2'-deoxy-2'-fluoroarabinofuranosyl analogs of the nusleoside antobiotics toyocamycin and sangivamycin. J. Med. Chem. 38:4106-4114, 1995.

Chen, X., Bastow, K., Goz, B., Kucera, L.S., Morris-Natschke, S.L., and Ishaq, K.S. Boronic acid derivatives targeting HIV-1. Antiviral Chem. Chemotherapy 7:108-114, 1996.

Chen, X., Bastow, K., Goz, B., Kucera, L.S., Morris-Natschke, S.L., and Ishaq, K. Synthesis and evaluation of novel thymidine analogs as antitumor and antiviral agents. J. Med. Chem. 39:3412-3417, 1996.

Ciacci-Woolwine, F., Kucera, LS., Richardson, SH., and Mizel, SB. Salmonells activate tumor necrosis factor alpha production in a human promonocytic call line via a relaesed polypeptide. Infect. Immun. (in press) 1997.

Porcari, AR., Devivar, RV., Kucera, LS., Drach, JC., and Townsend, LB. (1998) Design, synthesis and antiviral evaluations of 1-(substitutedbenzl)-2-substituted 5,6-dichlorobenzimidazoles as non-nucleoside analogs of 2,5,6-trichloro-1-(β -D-ribofuranosyl)benzimidazole. J. Med. Chem. 41: 1252-1262.

Kucera, LS., Moris-Natschke, SL., Chen, SY., Gumus, F., Ishaq, K., and Herrmann, DBJ. (1998) In vitro evaluation and characterization of newly designed alkylamidophospholipid analogues as anti-HIV-1 agents. Antiviral Chem. Chemotherapy 9: 157-165.

Ishaq,, K.., Morris-Natschke,, S.., Iyer,, N.., and Kucera,, L.. (1998) Acyclic nucleosides containing boronic acid target HIV-1.. 12th International AIDS Conference, Geneva (Switzerland), Clinical Science 2: 485-488.

Liu,, D-Z., Morris-Natschke,, S.L., Kucera,, L.S., Ishaq,, K.S., and Thakker,, D.R. (1999) Structure-activity relationships for enhancement of paracellular permeability by 2-alkoxy-3-alkylamidopropylphosphocholines across Caco-2 cell monolayers. Journal of Pharmaceutical Sciences 88: 1169-1174.

Kucera, G. L.., Goff, , C. L.., Iyer, , I.., Morris-Natschke, S.., Ishaq, K. S.., Wy-ick,, S. D.., Fleming, R. A.., and Kucera,, L. S.. (2001) Cellular metabolism in lymphocytes of a novel thioether-phospholipid-AZT conjugate with anti-HIV activity. Antiviral Res 50: 129-137.

**ABSTRACTS:**

Kucera LS, Goldberg HS, Goodman RN. Antibiotic-auxin interactions. Proc. Intersci. Conf. on Antimicrobial Agents and Chemotherapy. Am Soc Microbiol 1962:46.

Kucera LS, Herrmann EC Jr. Antiviral activity of unsubstituted purine. Bacteriol Proc 1966:114.

Kucera LS, Granoff A. Requirement for DNA synthesis of frog virus 3 (FV3). Bacteriol Proc 1967:158-159.

Kucera LS. Effect of temperature on initiation and regulation of frog virus 3 DNA replication. Bacteriol Proc 1969:181-182.

Simonson J, Kucera LS. Properties of amphibian cells transformed by co-cultivation with renal carcinoma (Lucke tumor) cells of Rana pipiens. Abstracts Annual Meeting Am Soc Microbiol 1973:205.

Melvin P, Kucera LS. Stimulation of host cell DNA synthesis by herpes simplex virus type 2 (HSV-2). Abstracts Annual Meeting Am Soc Microbiol 1974:241.

Kucera LS, Dodge WH. Interference of herpes simplex virus multiplication by avian oncornaviruses. IRCS Med Sci 1975;3(10 Suppl):24.

Marcon M, Kucera LS. The effect of temperature on DNA synthesis and infectious virus production in herpes simplex virus type 2 (HSV-2) infected human cells. Abstracts Annual Meeting Am Soc Microbiol 1975:239.

Kucera LS, Marcon M. Effect of temperature on stimulation of human cell DNA synthesis by herpes simplex virus type 2 (HSV-2). In: Bedson HS, et al, eds. Third Int Congress for Virology, Madrid, September 10-17, 1975: abstracts, 1975:239.

Kucera LS. Rapid selection of herpes simplex virus (HSV) induced oncogenic cells. Abstracts Annual Meeting Am Soc Microbiol 1976:204.

Heise ER, Kucera LS. Antibodies in human carcinoma to herpes simplex virus type 2 (HSV-2) induced antigens. Abstracts Annual Meeting Am Soc Microbiol 1976:217.

Marcon MJ, Kucera LS. Specificity of herpes simplex virus (HSV) stimulation of human embryonic fibroblast (HEF) cell DNA synthesis. Abstracts Annual Meeting Am Soc Microbiol 1976:219.

Kucera LS, Edwards I. Effect of in vitro cell passage on rat embryo fibroblasts transformed with photoinactivated herpes simplex virus (HSV). Abstracts Annual Meeting Am Soc Microbiol 1977:342.

Daniel L, Respess R, Kucera LS, Waite BM. Phospholipid synthesis in herpes simplex virus type 2 infected human embryo fibroblasts. J Supramol Struct 1979;3(Suppl):192.

Daniel L, Kucera LS, Respess R, Waite BM. Phospholipid synthesis in cells productively or abortively infected with herpes simplex virus type 2. Fed Proc 1979;38:627.

Waite BM, Morton RE, Daniel LW, Kucera LS, Hale AH. Polyunsaturated fatty acids of transformed cells. In: 11th International Congress of Biochemistry, Toronto, Canada, 1979: Abstracts. 1979:394.

Daniel LW, Kucera LS, Waite BM. Phospholipid acyl group composition of herpes simplex virus type 2 transformed rat cells. In: Nahmias AJ, Dowdle WR, Schinazi RF, eds. The human viruses - an interdisciplinary perspective. New York: Elsevier, 1981:635.

Kucera LS, Respess R, Edwards I. Cocarcinogen interactions with herpes simplex virus type 2 transformed rat embryo fibroblasts. In: Nahmias AJ, Dowdle WR, Schinazi RF, eds, The human herpesviruses - an interdisciplinary perspective. New York: Elsevier, 1981:636.

Respess RA, Kucera LS, Waite BM. Aminoacyl-fucosides as in vitro markers of tumorigenic potential in herpes simplex virus, type 2, (HSV-2) transformed rat cells. Abstracts Annual Meeting Am Soc Microbiol 1980;80:221.

Daniel LW, Waite BM, Kucera LS. Origin of phospholipids of purified herpes simplex virus, type 2. Abstracts Annual Meeting Am Soc Microbiol 1980:224.

Weeks BA, Kucera LS, Rapp HJ. 7,12-dimethylbenz(a)anthracene-induced tumors in guinea pigs: characterization and establishment in tissue culture. Abstracts Annual Meeting Am Soc Microbiol 1980:74.

Kucera LS, Furman P. Inhibition of herpes simplex virus type 2 transformed tumorigenic cell growth by acyclovir. In: Fifth Inter Congress of Virology, Strasbourg, France, August 2-7, 1981, abstracts. Paris: L'Imprimerie Centrale Commerciale, 1981:108.

Daniel LW, Kucera LS, Waite BM. Metabolism of fatty acids by cultured tumor cells and their diploid fibroblast precursors. Fed Proc 1980;39:1724.

Plunkett S, Heise ER, Ekstrand KE, Kucera LS, Hale AH, Witcofski RL, Raben M. A study of in vivo versus in vitro effects of irradiation on lymphocyte subpopulations: an analysis with reference to T-cells bearing Fc receptors. Int J Radiat Biol Phys 1981;7(Suppl):97.

Raben M, Heise ER, Kucera LS, Hale AH, Witcofski RL, Plunkett S. Effects of irradiation on lymphocyte subpopulations. In: Dubois JB, Serron B, Rosenfield C, eds. Immunopharmacologic effects of radiation therapy. New York: Raven Press, 1981:321-328.

Krebs CR, Kucera LS, Waite BM. Possible cofactor role of prostaglandin synthesis in herpes simplex virus type 2 (HSV-2) carcinogenesis. Med Ped Oncol 1984;12:76.

Flanders RT, Kucera LS, Ricardo MJ Jr, Plunkett S, Raben M. Characterization of an

HSV-2 tumor-associated antigen AG4 reactive with cervical carcinoma patients' sera. Med Ped Oncol 1984;12:79-80.

McCreedy BJ, Kucera LS, Ricardo MJ Jr. Fibronectin quantity and distribution is altered in lung tissue during the development of pulmonary fibrosis. Fed Proc 1984;43:1927.

Flanders RT, Kucera LS, Ricardo MJ Jr, Raben M. Characterization of HSV-2 tumor associated antigens ICPIO and ICP11/12 by Western blot analysis. Fed Proc 1984;43:595.

Krebs C, Waite M, Kucera LS. A cell-free assay for prostaglandin synthetase provides new evidence that the enzyme is transiently induced during herpes simplex virus type 2 (HSV-2) cell transformation. Abstracts VII International Congress of Virology, Edmonton, Alberta, Canada 1987;59.

Kucera L, Leake E, Raben D, Iyer N, Myrvik Q. Electron microscopic evidence for coinfection and replication of herpesvirus and HIV-1 in the same human T-cell. Abstracts. Vth International Conference on AIDS. Montreal, Quebec, Canada 1989:627.

Kucera L, Iyer N, Raben A, Modest E, Daniel L, Piantadosi C. Inhibition of HIV-1 plaque formation by a novel class of membrane-active ether lipid analogs. Abstracts. Vth International Conference on AIDS. Montreal, Quebec, Canada 1989;528.

Meyer KL, Morris-Natschke SL, Marasco CJ, Ishaq KS, Piantadosi C, Kucera LS. Synthesis and evaluation of anti-HIV-1 ether lipids. American Association of Pharmaceutical Scientists. Atlanta, GA, October 22-25, 1989.

Raben D, Kucera L, Iyer N, Ricardo M Jr, Leake E, Heise E, Myrvik Q. Transformed human T-cells coinfected with herpesvirus type 2 and human immunodeficiency virus. JAMA 1989;261:1359.

Pudlo, J.S., Drach, J.C., Kucera, L.S., Kahlon, J.B., Shannon, W.M., Townsend, L.B. Synthesis of branched cytidine and uridine analogs and activity against human immunodeficiency virus and herpesviruses. 30th Interscience Conference on Antimicrobial Agents and Chemotherapy. Atlanta, GA 1990.

Krugner, L., Iyer, N., Kucera, L. Granulocyte macrophage-colony stimulating factor stimulates persistent infected U1 cells to produce infectious human immunodeficiency virus (HIV). Abstract 78. 8th Annual Symposium on Nonhuman Primate Models for AIDS. Nov. 28-30, 1990. New Orleans, LA.

Drach, J.C., Townsend, L.B., Shipman, Jr., C., Wotring, L.L., Nassiri, M.R., Kucera, L.S., Buckheit, R.W., Shannon, W.M. Activity of triciribine and triciribine monophosphate against human immunodeficiency virus and cytotoxicity in human cells. Fourth N.C.D.D.G. Conference: Frontiers in HIV Therapy, San Diego, CA Nov. 3-7, 1991.

Krugner-Higby LA, Kucera LS, Goff DH, Edwards TV, Iyer N, Neufeld JA, Anand IS, Morris-Natchke SL, Piantadosi C. Novel membrane interactive ether lipid analogs inhibit HIV-1 glycoprotein interaction with CD4+ cells. The 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy; 1992 October 11-14: p. 164.

Chen X, Ishaq KS, Bastow K, Morris-Natschke SL, Kucera LS, Iyer N. Design and synthesis of novel nucleoside analogs as potential antiviral agents. American Association of Pharmaceutical Scientists. May 7, 1992: 41.

Kucera LS, Krugner-Higby, Iyer NP, Goff DH, Edwards TV, Neufeld JA, Anand IS, Puckett S, Morris-Natschke SL, Piantadosi C. Investigations on membrane active ether lipid analogs that alter functional expression of HIV-1 induced glycoproteins and inhibit pathogenesis. J. Cellular Biochemistry 1993; Supplement 17E:16.

Kucera LS, Iyer N, Morris-Natschke S, Piantadosi C. Novel membrane active ether lipids that inhibit HIV-1 induced gp160/120 functional expression and pathogenesis. Abstract Book. IXth International Conference on AIDS in affiliation with the IVth STD World Congress; 1993 June 6-11: Abst. No. PO-A25-0578, p231.

Kucera L, Fowler C, Iyer N, Whang C, Morris-Natschke S, Ishaq K, Piantadosi C. Novel ether lipid analogues of platelet activating factor with anti-hepatitis B virus activity. Abstract Book. 34th Interscience Conference on Antimicrobial Agents and Chemotheraphy; 1994 October 4-7: Abstract No. H125, p. 257.

Morris-Natschke SL, McLean JW, Ishaq KS, Kucera LS. Novel lipid analogues as potential anti-HIV and antitumor agents. Abstract. American Association of Pharmaceutical Scientists. San Diego, CA, October 6-10, 1994, Pharmaceutical Research 1994 11:S120.

Thompson, J., Iyer, N.P., Poduval, R., Ishaq, K., Morris-Natschke, S., and Kucera, L.S. Phospholipid analog inhibition of human immunodeficiency virus envelope glycoprotein-mediated cell fusion. Abstracts of the second National Conference on Human Retroviruses. American Society for Microbiology, Washington, D.C. January 29 – February 2, 1995.

Thompson, J., Iyer, N.P., Poduval, R., Ishaq, K., Morris-Natschke, S., and Kucera, L.S. Phospholipid analog inhibition of human immunodeficiency virus envelope glycoprotein-mediated cell fusion. 2nd National Conference on Antiviral Research, Santa Fe, NM; 1995, April 23 – 28, Abstract 63, p28. Abstracts published in Antiviral Research 26:A260, 1995.

Chen, X., McLean, J.W., Morris-Natschke, S.L., Goz, B., Ishaq, K., Iyer, N., and Kucera, L.S. Acyclic nucleoside analogs containing the boronic acid moiety as potential antiviral agents targeting Hiv-1. Abstracts of the 35th Interscience conference on Antimicrobial Agents and Chemotherapy. Moscone Center, San Francisco, CA; Abstract I145, p230.

September 17-20, 1995.

Ciacci, F., Richardson, S.H., Kucera, L.S., Iyer, N., and Mizel, S.B. A secreted salmonella protein upregulates TNFa expression in a human promonocytic cell line. Abstracts. 96th General Meeting of the American Society for Microbiology, May 19-23, 1996, Ernest N. Morial Convention Center, New Orleans, LA, Abstract B-144, p179.

Ishaq, K., McLean, J.W., Morris-Natschke, S.L., Iyer, N., and Kucera, L.S. Synthesis and biological evaluatio of cyclic phospholipid analogues targeting HIV-1. Abstract. XI International Conference on AIDS. Vancouver, BC, July 7-12, 1996. Abstract Mo.A.1095, p71.

Herrmann, D.B.J., Kucera, L.S., Zilch, H., Mertans, A., and Opitz, H.G. Bm 21.1290: In-vitro evaluation of a potential new anti-AIDS compound. Abstract XI International Conference on AIDS. Vancouver, BC, July 7-12, 1996. Abstract Mo.A. 1057, p64.

Kucera, L.S., Iyer, N., Ishaq, K., Morris-Natschke, S.L., and Herrmann, D.B.J. Characterization of novel amidophospholipid (CP-51) anti-HIV-1 activity and mechanism of action. Abstracts. 4th Conference on Retroviruses and Opportunistic Infections. January 22-26, 1997, Washington, DC. Abstract # 157, p93.

Kucera, L.S., Iyer, N., Morris-Natschke, S., Ishaq, K.S., and Herrmann, D.B.J. In-vitro evaluation and characterization of newly designed alkylamidophospholipid (INK) analogues as anti-HIV-1 agents. Abstract. New Opportunities for HIV Therapy-From Discovery to Clinical Proof-of-Concept. 2nd Joint Conference of the Strategic Program for Innovative Research on AIDS Treatment (SPIRAT) and the National Cooperative Drug Discovery Groups for the Treatment of HIV Infection (NCDDG-HIV). June 22-16, 1997, Vienna, VA.

Herrmann, D.B.J., Opitz, H.G., and Kucera, L.S. BM 21.1290: Evaluation of the antiretroviral activity of a new anti-AIDS drug in-vivo. Abstract XI International Conference on AIDS. Vancouver, BC, July 7-12, 1996. Abstract # 149, p92.

Herrmann, D.B.J., Opitz, H.-G., Kucera, LS. In vitro and in vivo evaluation of the anti-AIDS compound fozivudine. Absrtacts #174 of the Fall Meeting of the Deutsche Gesellschaft fur Experimentelle und klinische Pharmakologie und Toxikologie. September 14-17, 1997, Wien, Austria, Archives of Pharmacology, No. 1, Vol. 356, 1997.

Kucera, L.S., Iyer, N., Herrmann, D., and Opitz, H. MIP-1a , MIP-1b and IL-16 anti-HIV activity. Abstracts. American Society for Virology 16th Annual Meeting, Montana State University, Bozman, MT, July 19-23, 1997. Abstract # W17-3, p124.

Morris-Natschke, S.L., Chen, S. Y., Ishaq, K.S., Gumus, F., Iyer, N. and Kucera, L.S. Design of selective phospholipids as anti-HIV-1 agents. Abstract. Southeastern Regional Lipid Conference, Cashiers, NC Nov. 5-7, 1997.

Kucera, L.S. Evaluation of novel synthetic ether lipids with selective activity against HIV-1 and hepatitis B virus. Abstract. Connectivity Expo 2001. Winston-Salem, NC April, 2001.

Alexander, R.L., Morris-Natschke, S.L., Ishaq, K.S., Kucera, L.S., and Kucera, G.L. Abstracts. Novel phospholipid gemcitabine (Phosgem) and Ara-C conjugates for increasing uptake and bypassing resistance mechanisms expressed by tumor cells. Southeastern Regional Lipid Conference, Cashiers, NC Nov. 1-3, 2001

**Letters:**

Gusdon JP Jr, Kucera LS. The oncogenic potential of dye-light treatment of herpetic lesions. Am J Obstet Gynecol 1974;120:569-570.

Kucera LS. In vitro propagation of clinical isolates. AIDS Research and Reference Reagent Program Courier No. 90-03, November 1990, p 5.

Kucera LS. Letters to the editor. Research is part of health care. Winston-Salem Journal, September 19, 1994.

Kucera, LS. Letters to the editor. Don't cut funds for medical research. Winston-Salem Journal, July 2, 1995.

Antiviral Chemistry & Chemotherapy 9: 157–165

# *In vitro* evaluation and characterization of newly designed alkylamidophospholipid analogues as anti-human immunodeficiency virus type 1 agents

LS Kucera[1], N Iyer[1], SL Morris-Natschke[2], SY Chen[2], F Gumus[3], K Ishaq[2]* and DBJ Herrmann[4]

[1]Wake Forest University School of Medicine, Winston-Salem, N.C., USA
[2]School of Pharmacy, University of North Carolina, Chapel Hill, N.C., USA
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Etiler, Ankara, Turkey
[4]Boehringer Mannheim, Mannheim, Germany

*Corresponding author: Tel: +1 919 962 0065; Fax: +1 919 966 6919; E-mail: kishaq.pharm@mhs.unc.edu

**Our laboratories first reported two novel classes of complex synthetic lipids, including alkylamidophosphocholines (PC lipid; CP-51) and alkylamidophosphate ester-linked lipid–AZT conjugates (lipid–AZT conjugates; CP-92), with selective and potent activity against human immunodeficiency virus type 1 (HIV-1). To extend these observations, we synthesized additional PC lipids and lipid–AZT conjugates (INK and INK–AZT conjugate) to evaluate their structure–activity relationships by testing for selectivity against infectious wild-type (wt) and drug-resistant HIV-1 replication, virus fusogenic activity and toxicity for mouse bone marrow cells. PC lipid compounds with medium chain lengths at positions 1 and 2 gave an improved selective index (SI). INK-3, with 12 and 8 carbons and INK-15, with 10 and 12 carbons were among the most selective when evaluated in CEM-SS cells. INK-14, a lipid–AZT conjugate where AZT replaced the choline in PC lipid INK-3, gave the highest SI of >1250 against both infectious wt HIV-1 replication in CEM-SS cells and a clinical isolate in peripheral blood leukocytes. Notably, the PC lipid compounds INK-3 and INK-15, but not the lipid–AZT conjugate INK-14, were potent inhibitors of matched pairs of AZT-sensitive and AZT-resistant HIV-1 clinical isolates. INK-3 also inhibited replication of HIV-2 and TIBO-resistant HIV-1, and inhibited HIV-1-mediated fusogenic activity by 78, 41 and 9% in a dose-dependent manner. The $TC_{50}$ for mouse bone marrow cells was >100 μg/ml for INK-3 compared to 9.15–14.17 μg/ml for CP-51 and 0.142–0.259 μg/ml for AZT. These data suggest that optimum PC lipid compounds are significantly less toxic than AZT and have high potential as novel therapeutic agents for AIDS.**

Keywords: alkylamidophosphocholine; phospholipid–nucleoside conjugate; HIV-1; AIDS

## Introduction

Our laboratories first reported on two novel classes of complex synthetic lipids (alkylamidopropyl or alkylthioglycerol phosphocholines and phosphate ester-linked lipid–AZT conjugates) that have selective and potent activity against infectious human immunodeficiency virus (HIV) replication and pathogenesis *in vitro*. These compounds have a unique site of action involving alteration of HIV gp160/gp120 activity and production of defective virus particles (Kucera *et al.*, 1990a,b; Meyer *et al.*, 1991; Piantadosi *et al.*, 1991; Krugner-Higby *et al.*, 1995). A thiolipid–nucleotide conjugate (BM21-1290) has recently been introduced into Phase I/II tolerability and efficacy trials in ARC/AIDS patients by Boehringer Mannheim (Herrmann DBJ, Schleger C & Opitz HG; Antiretroviral activity of the novel candidate anti-AIDS drug BM21.1290 in the Friend-virus leukemia system in vivo; *VIth German AIDS-Congress*, October 24–26 1997, Munich, Germany, Abstract V160; Herrmann DBJ, Opitz HG & Kucera LS; BM21-1290: evaluation of the anti-retroviral activity of a new anti-AIDS drug in-vivo. *IVth Conference on Retroviruses and Opportunistic Infections*, January 22–26 1997, Washington, DC, USA, Abstract p92). Among the reported alkylamidopropyl phosphocholine (PC lipid) analogues synthesized and tested for anti-HIV-1 activity, the most selective was compound CP-51, 1-octadecanamido-2-ethoxypropyl-3-phosphocholine (Kucera *et al.*, 1990a). In order to extend these observations, we synthesized a series of CP-51 analogues to

evaluate their structure–activity relationships and thus to optimize the PC lipid compound for future evaluation as a potential therapeutic agent for AIDS. Results of this investigation indicated that among the PC lipid compounds tested in CEM-SS cells, compounds INK-3 and INK-15 with intermediate hydrocarbon chain lengths of 12 and 10, respectively, at position 1, and of 8 and 12, respectively, at position 2, gave the highest selectivity index (SI) against infectious HIV-1 replication. Selected PC lipid compounds were active against AZT-resistant clinical isolates, TIBO-resistant HIV-1 and wild-type HIV-2, inhibited HIV-1 fusogenic activity, and were less toxic to mouse bone marrow cells compared to AZT. Based on these interesting results, optimum PC lipid compounds may have great potential as novel therapeutic agents for AIDS, either alone or in combination therapy.

## Materials and Methods: Chemistry

All chemicals were used as provided by the supplier without further purification unless otherwise indicated. AZT was obtained from Boehringer Mannheim (Mannheim, Germany). Column chromatography was performed with silica gel 60 (230–400 or 70–230 mesh). Melting points were obtained on a Hoover Meltemp apparatus and are uncorrected. Proton NMR spectra were obtained on Bruker 300 MHz or Varian 400 MHz spectrometer as solutions in $CDCl_3$ with $Me_4Si$ as an internal standard. The final compounds were, in general, hygroscopic solids. FAB mass spectra were run on a VG 70S mass spectrometer. Thin layer chromatography (TLC) of phosphocholines in $CHCl_3$ : MeOH : $NH_4OH$ (70 : 35 : 7) and $CHCl_3$ : MeOH (2 : 1) gave a single spot, which gave a blue colour with a modified Dittmer–Lester reagent (Ryu & MacCoss, 1979). A representative synthetic procedure is given for INK-3; the remaining phosphocholines were prepared from the appropriate reagents using analogous procedures as those detailed below and in our previous papers (Piantadosi *et al.*, 1991; Meyer *et al.*, 1991; Morris-Natschke *et al.*, 1993). Complete high resolution mass spectra results are available as supplementary data from the Editor-in-Chief.

### 3-Dodecanamido-1,2-propanediol

3-Amino-1,2-propanediol (42 g, 0.47 mol) was dissolved in pyridine (150 ml) and DMF (200 ml). Dodecanoyl chloride (47 g, 0.46 mol) in 150 ml DMF was added dropwise. After 24 h at room temperature, the product was removed by filtration, washed with $H_2O$ and recrystallized from MeOH, then from $CHCl_3$. The amide was obtained in 28% yield (34.7 g, 0.013 mol, m.p. 94–96°C). $^1$H NMR ($CDCl_3$): 0.90 (t, 3H, terminal $CH_3$), 1.25 [m, 16H $(CH_2)_8$], 1.6 (m, 2H, NHCOCH$_2$*CH$_2$*), 2.25 (t, 2H, NHCO*CH$_2$*), 3.45 (m, 2H, *CH$_2$*NH), 3.6 (m, 2H, *CH$_2$*OH), 3.8 (m, 1H, *CH*OH), 5.8 (t, 1H, NH).

### 3-Dodecanamido-1-triphenylmethoxy-2-propanol

The above diol (5.5 g, 0.020 mol) was dissolved in dry pyridine (50 ml), then a solution of trityl chloride (5.6 g, 0.020 mol) in pyridine (50 ml) was added dropwise at 50°C. The reaction mixture was stirred at this temperature for 10 h and then cooled. The pyridine was removed under vacuum, and water (100 ml) was added to the solid residue. After filtration and washing, the crude product was partitioned between water and $CHCl_3$. The water layer was extracted again with $CHCl_3$ and the organic layers combined and dried over anhydrous $Na_2SO_4$. After removing solvent, the resulting solid was dissolved in hexane : ethyl acetate (3 : 1); 1 g of starting diol was filtered. The mother liquor was concentrated and chromatographed on silica gel with a discontinuous gradient of hexane : EtOAc to give 7 g (0.014 mol, 70% yield) of pure product. $^1$H NMR ($CDCl_3$): 0.90 (t, 3H, terminal $CH_3$), 1.25 [m, 16H $(CH_2)_8$], 1.6 (m, 2H, NHCOCH$_2$*CH$_2$*), 2.2 (t, 2H, NHCO*CH$_2$*), 3.2–3.4 (overlapping m, 3H, *CH$_2$*NH and *CH$_a$*CH$_b$OTr), 3.55 (m, 1H, CH$_a$*CH$_b$*Tr) 3.9 (m, 1H, *CH*OH), 5.7 (t, 1H, NH), 7.3–7.5 (m, 15H, aromatic H).

### 3-Dodecanamido-2-octyloxy-1-triphenylmethoxypropane

The synthesized trityl ether (7.0 g, 0.013 mol) in 30 ml dry THF was added dropwise to an ice-cooled suspension of sodium hydride (1.0 g of 60% oil dispersion, 0.025 mol) in 100 ml THF under nitrogen. After warming to room temperature, heat was applied (60°C) for 1 h. 1-Bromooctane (2.9 g, 0.015 mol, neat) was added dropwise and heating continued for 10 h. After cooling, ice and then water were added slowly. The biphasic system was separated and the water layer extracted with EtOAc (3×50 ml). All organic fractions were combined and dried over anhydrous $Na_2SO_4$. After removing solvent *in vacuo*, the residue was chromatographed with a gradient of hexane : EtOAc (100 : 0 to 6 : 1) to give 3.6 g (38% yield) of pure product. Impure product was rechromatographed using the same conditions to give an added 3.0 g (31% yield) of product as a viscous oil. $^1$H NMR ($CDCl_3$): 0.85 (t, 6H, terminal $CH_3$), 1.25 [m, 26H $(CH_2)_8$ and $(CH_2)_5$], 1.45 (m, 4H, NHCOCH$_2$*CH$_2$* and OCH$_2$*CH$_2$*), 2.2 (t, 2H, NHCO*CH$_2$*), 3.2–3.6 (overlapping m, 7H, CH$_2$CHCH$_2$, OCH$_2$), 5.75 (t, 1H, NH), 7.3–7.5 (m, 15H, aromatic H).

### 3-Dodecanamido-2-octyloxy-1-propanol

Detritylation of the above compound (6.0 g, 9.5 mmol) was accomplished using p-toluenesulphonic acid (0.6 g, 3.1 mmol) in $CHCl_3$ : MeOH (60 ml : 18 ml). The reaction

mixture was stirred at room temperature for 24 h then saturated $NaHCO_3$ solution was added and stirred for 30 min. The layers were separated and the $CHCl_3$ fraction dried over anhydrous $Na_2SO_4$. After concentration *in vacuo*, the residue was purified twice by column chromatography using $CHCl_3$ : MeOH as eluent (100 : 0 and 15 : 1) to give 3.0 g (80% yield) of pure 3-dodecanamido-2-octyloxy-1-propanol (m.p. 42–43°C). $^1$H NMR ($CDCl_3$): 0.9 (t, 6H, terminal $CH_3$), 1.25 [m, 26H $(CH_2)_8$ and $(CH_2)_5$], 1.6 (m, 4H, $NHCOCH_2\mathit{CH_2}$ and $OCH_2\mathit{CH_2}$), 2.2 (t, 2H, $NHCO\mathit{CH_2}$), 3.2–3.7 (overlapping m, 7H, $CH_2CHCH_2$, $OCH_2$), 5.75 (t, 1H, NH).

### 3-Dodecanamido-2-octyloxypropyl 2-bromoethyl phosphate

3-Dodecanamido-2-octyloxy-1-propanol (1.0 g, 2.6 mmol) in 60 ml 2 : 1 anhydrous $Et_2O$ : THF was cooled to 0°C. Pyridine (3.3 g) then 2-bromoethyl dichlorophosphate (2.4 g, 0.010 mol, prepared as described by Hansen *et al.*, 1992) were added dropwise. After warming to room temperature, the mixture was refluxed for 4 h, cooled and water (10 ml) added. After stirring for 30 min, the solvent was removed *in vacuo* and the residue dissolved in 100 ml $CHCl_3$ : MeOH (2 : 1). The solution was extracted with water and back-extracted with 2×50 ml 2 : 1 $CHCl_3$ : MeOH. The combined organic fractions were dried over anhydrous $Na_2SO_4$, evaporated and chromatographed on silica gel using a gradient of $CHCl_3$ : MeOH (100 : 1 to 15 : 1). The desired bromoethylphosphate (633 mg, 1.1 mmol) was obtained in 43% yield. $^1$H NMR ($CDCl_3$): 0.85 (t, 6H, terminal $CH_3$), 1.25 [m, 26H $(CH_2)_8$ and $(CH_2)_5$], 1.55 (m, 4H, $NHCOCH_2\mathit{CH_2}$ and $OCH_2\mathit{CH_2}$), 2.3 (t, 2H, $COCH_2$), 3.4–3.7 (overlapping m, 7H, $\mathit{CHCH_2}NHCO$, $CH_2Br$, $OCH_2$), 3.85 and 4.25 (two m, 4H, $CH_2OPO_3CH_2$), 6.8 (m, 1H, NH).

### 3-Dodecanamido-2-octyloxypropyl phosphocholine (INK-3)

The above phosphate ester (633 mg, 1.1 mmol) in 52 ml $CHCl_3$ : isopropanol : DMF (5 : 3 : 5) was reacted with aqueous $Me_3N$ (7.6 ml) at 65°C for 5 h. After cooling, $Ag_2CO_3$ (380 mg) was added and the heat reapplied for 1 h. After filtering the precipitated AgBr, the solvent was removed *in vacuo* and the residue purified by chromatography on silica gel using $CHCl_3$ : MeOH (10 : 1 to 2 : 1) followed by $CHCl_3$ : MeOH : $NH_4OH$ (75 : 25 : 5) to give 275 mg pure (46% yield) and 140 mg impure phosphocholine. FAB MS showed a $[MH]^+$ ion at 551.418345 ($C_{28}H_{60}N_2O_5P$, 1.0 p.p.m.). $^1$H NMR ($CDCl_3$): 0.87 (t, 6H, terminal $CH_3$), 1.25 [m, 26H $(CH_2)_8$ $(CH_2)_5$], 1.5 (m, 4H, $NHCOCH_2\mathit{CH_2}$, $OCH_2\mathit{CH_2}$), 2.2 (t, 2H, $COCH_2$), 3.3 [s, 9H, $N(CH_3)_3$], 3.3–3.8 (overlapping m, 5H, $CH_2CHOCH_2$) (3.8–3.9, overlapping m, 4H, $CH_2OP$, $\mathit{CH_2}NMe_3$), 4.4 (m, 2H, $PO_3CH_2$), 7.0 (m, 1H, NH).

### 3-Dodecanamido-2-octyloxypropyl diphenyl phosphate

Diphenylchlorophosphate (0.4 ml, 1.7 mmol) in 10 ml diethyl ether was cooled to 4°C under nitrogen. 3-Dodecanamido-2-octyloxy-1-propanol (500 mg, 1.3 mmol) in 8 ml pyridine and 10 ml $Et_2O$ was added dropwise. The solution was warmed to room temperature, then heated to around 52°C for 4 h and cooled to room temperature, diluted with 30 ml $Et_2O$, washed with 15 ml portions of water, 0.5 M HCl and water. The organic layer was dried over anhydrous $Na_2SO_4$, filtered and concentrated *in vacuo* to an oil. Chromatography with a gradient of hexane : EtOAc (10 : 1 to 3 : 1) gave 556 mg (0.9 mmol, 69%) of pure product. $^1$H NMR ($CDCl_3$): 0.9 (t, 6H, terminal $CH_3$), 1.25 [m, 26H $(CH_2)_8$ and $(CH_2)_5$], 1.55 (m, 4H, $NHCOCH_2\mathit{CH_2}$ and $OCH_2\mathit{CH_2}$), 2.3 (t, 2H, $COCH_2$), 3.3–3.7 (overlapping m, 5H, $OCH_2$, $\mathit{CHCH_2}NHCO$), 4.25 (m, 2H, $CH_2OP$), 5.9 (m, 1H, NH), 7.2–7.8 (m, 10H, aromatic H).

### 3-Dodecanamido-2-octyloxypropyl phosphatidic acid

$PtO_2$ (81 mg) was placed in a Parr hydrogenation bottle. 3-Dodecanamido-2-octyloxypropyl diphenyl phosphate (589 mg, 1.0 mmol) in 118 ml EtOH was then added. After hydrogenation at 158.5 kPa (23 p.s.i.) for 4.5 h, the reaction mixture was filtered and checked for completion by TLC. To ensure complete conversion to the phosphatidic acid, hydrogenation was continued for another 6 h using 120 mg of fresh catalyst. The reaction mixture was then filtered through Celite and the EtOH removed *in vacuo*. The residue was chromatographed on silica gel using 4 : 1 $CHCl_3$ : MeOH as eluent to obtain 233 mg (0.54 mmol, 54% yield) of pure phosphatidic acid. $^1$H NMR ($CDCl_3$): 0.9 (t, 6H, terminal $CH_3$), 1.25 [m, 26H $(CH_2)_8$ and $(CH_2)_5$], 1.55 (m, 4H, $NHCOCH_2\mathit{CH_2}$ and $OCH_2\mathit{CH_2}$), 2.3 (t, 2H, $COCH_2$), 3.3–3.7 (overlapping m, 5H, $OCH_2$, $\mathit{CHCH_2}NHCO$), 4.0 (m, 2H, $CH_2OP$), 6.8 (m, 1H, NH).

### 3′-Azido-3′-deoxy-5′-(3-dodecanamido-2-octyloxypropyl)-phosphothymidine (INK-14)

The procedure of Piantadosi *et al.* (1991) was followed to synthesize INK-14 in 22% yield from the above phosphatidic acid and AZT. FAB MS showed a $[MH+Na]^+$ ion at 737.403800 ($C_{33}H_{59}N_6O_9PNa$, −8.0 p.p.m.). $^1$H NMR ($CDCl_3$): 0.9 (t, 6H, terminal $CH_3$), 1.25 [m, 26H $(CH_2)_8$ and $(CH_2)_5$], 1.55 (m, 4H, $NHCOCH_2\mathit{CH_2}$ and $OCH_2\mathit{CH_2}$), 1.8 (s, 3H, thymine $CH_3$), 2.2 (t, 2H, $COCH_2$), 2.3–2.55 (m, 2H, 2′-$CH_2$), 3.3–3.6 (overlapping m, 5H, $OCH_2$, $\mathit{CHCH_2}NHCO$), 3.8–4.2 (overlapping m, 5H, $CH_2OPO_3CH_2$, 4′-CH), 4.5 (1H, 3′-CH), 6.15 (m, 1′-CH), 7.4 (m, 1H, thymine CH).

## Materials and Methods: Virology

### Virus stocks

HIV-1 (strain IIIB) was propagated in H9IIIB cells as previously described (Krugner-Higby *et al.*, 1995). Stocks of HIV-2 from R Weiss (Institute of Cancer Research, London, UK), TIBO-resistant HIV-1 (RT mutant at codons 103 and 181) from E Emini (Merck, Sharp & Dohme Research Laboratories, West Point, Pa., USA) and AZT-resistant HIV-1 isolates 1073 (RT mutant at codons 70 and 215) and 1074 (RT mutant at codons 70 and 215), and matched pairs of AZT-sensitive and AZT-resistant HIV-1 clinical isolates G-762 (wild-type), G-691 (RT mutant) and H112-2 (wild-type), G-910 (RT mutant at codon 215) from D Richman (University of California, La Jolla, Calif., USA) were obtained through the AIDS Research and Reference Reagent Program (Division of AIDS Program, NIH, Bethesda, Md., USA). Stocks of viruses were propagated in acutely infected CEM-SS cells as previously described (Krugner-Higby *et al.*, 1995).

### Cell cultures

All primary peripheral blood leukocytes (PBL) and cell lines were incubated and maintained in RPMI 1640 medium supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 2 mM L-glutamine and 20% fetal bovine serum (growth medium) as previously described (Krugner-Higby *et al.*, 1995). PBL were obtained from HIV-1-seronegative male and female personnel in the laboratory. Whole blood was separated into PBL using Isolymph (Gallard-Schlesinger Industries, Carle Place, N.Y., USA) according to a protocol supplied by the manufacturer. The separated PBL were activated for 3 days in the presence of phytohaemagglutinin (PHA; 5 μg/ml in growth medium), washed in growth medium, treated with polybrene (5 μg/ml in growth medium) and infected with HIV-1 in the presence of polybrene (1 μg/ml in growth medium). After a 2 h virus attachment, the cells were washed with growth medium to remove unattached HIV-1 and incubated ($1\times10^6$ cells/ml of growth medium) for 96 h with or without added test compounds. Supernatant medium was harvested to measure virus replication by reverse transcriptase (RT) activity (Krugner-Higby *et al.*, 1995).

### $TC_{50}$ determinations

To measure the cytotoxicity in CEM-SS cells, test compounds were dissolved in 95% ethanol, methanol : chloroform (1 : 1) or RPMI 1640 plus 20% FBS growth medium, diluted in $\log_{10}$ or 0.5 $\log_{10}$ series in growth medium and tested for cytotoxicity in triplicate wells containing 10 000 CEM-SS cells/well of a 96-well plate. A viable cell count was made prior to the test to ensure more than 90% cell viability. Log phase cells were treated with test compound for 48 h at 37°C (approximately 2.5 cell generations in untreated control cultures) and pulse-labelled with 1 μCi [$^3$H]TdR (sp. act. 20 Ci/mmol) for 6 h before harvesting the cells using a multichannel cell harvester to measure total DNA synthesis in the presence or absence of compound. To measure cytotoxicity in PBL, the cells were activated with PHA (5 μg/ml) for 72 h, washed to remove the PHA and 20 000 activated PBL cells/well were cultured for 96 h in growth medium with or without test compound as described for CEM-SS cells. The treated PBL cells were labelled with 0.1 μCi [$^3$H]TdR (sp. act. 20 Ci/mmol) during the final 24 h culture period before harvesting. From the data, a $TC_{50}$ for cytotoxicity was calculated as previously described by Chou and coworkers (Chou & Talalay, 1987; Johnson *et al.*, 1989) as cited by Piantadosi *et al.* (1991).

### Syncytium plaque assay

The syncytium plaque assay for infectious virus multiplication was done in the presence or absence of PC lipid or lipid–AZT conjugate as previously described by us (Kucera *et al.*, 1990a). The syncytium plaques were counted on day 5 or 6 post-infection and treatment and the percentage inhibition and effective concentration$_{50}$ ($EC_{50}$) were calculated by the method of Chou and coworkers (Chou & Talalay, 1987; Johnson *et al.*, 1989) as cited by Piantadosi *et al.* (1991).

### Fusogenic assay

Persistently HIV-1-infected H9IIIB cells were treated with compound for 96 h and cocultured with uninfected CD4$^+$ CEM-SS cells (ratio 1 : 100) in the presence of added compound for 48 h. The number of fusogenic cells was counted relative to untreated control cells to determine the percentage inhibition of fusogenic cells. The $EC_{50}$ values were calculated by the method cited by Piantadosi *et al.* (1991).

### Colony forming units–granulocyte macrophage (CFU-GM) assay

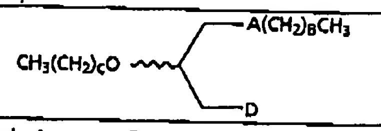
Mouse bone marrow granulocyte/macrophage cells were harvested and suspended in soft agar medium with or without added test compound (Herrmann DBJ, Kucera LS, Zilch H, Mertens A & Opitz HG; BM21.1290: in-vitro evaluation of a potential new anti-AIDS compound; *XIth International Conference on AIDS*, July 7–12 1996, Vancouver, Canada, Abstract p64). After incubation, the number of cell colonies was counted to determine the percentage inhibition of colony formation relative to a mock (PBS)-treated control. The 50% toxic concentration ($TC_{50}$) was calculated by the method cited by Piantadosi *et al.* (1991).

## Results

### Chemistry

Phospholipid and lipid–AZT conjugates were synthesized in our laboratories as previously described (Piantadosi *et al.*, 1991). Briefly, 3-amino-1,2-propanediol was reacted with an acyl chloride to form an alkylamidopropanediol. The primary alcohol was protected as the trityl ether by reaction with trityl chloride, then the secondary alcohol was alkylated with the appropriate alkyl halide using sodium hydride as the base. After removing the trityl ether with *p*-toluenesulphonic acid, the hydroxy group was converted either to a phosphocholine [reaction first with bromoethyl phosphodichloridate (Hansen *et al.*, 1982) then with aqueous trimethyl amine] or, in the case of INK-14, to the phosphatidic acid (reaction with diphenyl chlorophosphate then catalytic hydrogenolysis over $PtO_2$). The phosphatidic acid was coupled with AZT using dicyclohexylphosphodiimide as the condensing agent.

**Table 1. Chemical structures of synthetic phospholipids**




| Compound | A | B | C | D* |
|---|---|---|---|---|
| CP-51 | NHCO | 16 | 1 | PC |
| INK-1 | NHCO | 10 | 11 | PC |
| INK-2 | NHCO | 10 | 9 | PC |
| INK-3 | NHCO | 10 | 7 | PC |
| INK-4 | O | 11 | 9 | PC |
| INK-6 | NHCO | 10 | 5 | PC |
| INK-7 | NHCO | 16 | 7 | PC |
| INK-8 | NHCO | 10 | 7 | $OPO_3(CH_2)_3N(CH_3)_3$ |
| INK-13 | NHCO | 8 | 7 | PC |
| INK-14 | NHCO | 10 | 7 | $OPO_3AZT$ |
| INK-15 | NHCO | 8 | 11 | PC |

*PC, $OPO_3CH_2CH_2N(CH_3)_3$.

### Evaluation of compounds for cytotoxicity and anti-HIV-1 activity

To optimize the activity and/or SI, PC lipid and lipid–AZT conjugate compounds varying in hydrocarbon chain lengths at position 1 or 2 were synthesized (Table 1) and evaluated for cytotoxicity, anti-HIV-1 activity and SI in CEM-SS and PBL cells. Results of these experiments (Table 2) using CEM-SS cells indicated that the $TC_{50}$ ranged from 27.6 (INK-2) to >100 μM (INK-13 and -14). The $EC_{50}$ ranged from <0.08 (INK-14) to 1.02 μM (INK-8). The SI ranged from 53.2 (INK-8) to >1250 (INK-14) (Table 2). Compounds with a 12 or 10 carbon chain length at position 1, an 8 or 12 carbon chain length at position 2, and phosphocholine at position 3 of the three carbon backbone (INK-3 or INK-15) gave the highest SI (298.9 and 389.0, respectively) among the PC lipid compounds. INK-14, which has the same hydrocarbon chain lengths at position 1 and position 2 as INK-3 but is linked at position 3 through a phosphate ester bond to AZT in place of phosphocholine (see Table 1), gave the highest SI (>1250)

**Table 2. Evaluation of PC lipid and lipid–AZT conjugate compounds for cell cytotoxicity, anti-HIV-1 activity and SI in CEM-SS and PBL cells***

| | $TC_{50}$ (μM)† | | $EC_{50}$ (μM)† | | SI‡ | |
|---|---|---|---|---|---|---|
| Compound | CEM-SS | PBL | CEM-SS | PBL | CEM-SS | PBL |
| INK-1 | 30.3±8.9 | 43.7 | 0.47±0.2 | 1.25 | 64.5 | 34.9 |
| INK-2 | 27.6+5.7 | 48.4 | 0.15±0.1 | >3 | 184.0 | <16.1 |
| INK-3 | 56.8±22.4 | >50 | 0.19±0.1 | >3 | 298.9 | <16.7 |
| INK-4 | 75.7±21.7 | 48.2 | 0.64±0.4 | >3 | 118.3 | <16.1 |
| INK-6 | 40.2±12.8 | >50 | 0.21±0.1 | 0.63 | 191.4 | >79.4 |
| INK-7 | 36.0 | 31.6 | 0.67 | 1.5 | 53.7 | 21.0 |
| INK-8 | 54.3±14.3 | >50 | 1.02±0.3 | >3 | 53.2 | <16.7 |
| INK-13 | >100±0.0 | >50 | 0.77±0.7 | 2.5 | >129.9 | >20.0 |
| INK-14 | >100±0.0 | >50 | <0.08±0.08 | 0.62 | >1250 | >80.6 |
| INK-15 | 38.9±2.9 | 42.1 | 0.10±0.06 | >3 | 389.0 | <14.0 |
| CP-51§ | 14.3±2.3 | ND | 0.11±0.06 | ND | 130 | – |
| AZT§ | 3.7±1.6 | ND | 0.009±0.008 | ND | 411 | – |

*The $TC_{50}$ (cytotoxicity) and $EC_{50}$ (anti-HIV-1 activity) data from CEM-SS cells represent averages ±SD of 2–4 independent experiments per compound except for compound INK 7, which was tested once. Data from PBL cells were from a single independent experiment. To determine the $TC_{50}$, five serial concentrations of each compound were assayed in triplicate for [$^3$H]TdR incorporation into total DNA. To determine the $EC_{50}$, three to five serial concentrations of each compound were assayed in duplicate. The syncytial plaque assay was used with CEM-SS cells infected with the prototype of HIV-1 (strain H9IIIB). A reverse transcriptase assay was used with PBL cells infected with a clinical isolate of HIV-1 (strain H112-2) as cited in Kucera *et al.* (1990a).
†$TC_{50}$ and $EC_{50}$ values were calculated by the method of Chou and coworkers (Chou & Talalay, 1987; Johnson *et al.*, 1989) as cited by Piantadosi *et al.* (1991).
‡SI is the ratio $TC_{50}/EC_{50}$.
§Data from Piantadosi *et al.* (1991).

Table 3. Evaluation of PC lipids INK-3 and CP-51 and AZT against TIBO-resistant, AZT-resistant HIV-1 and wild-type HIV-2 measured by syncytial plaque assay

| | $EC_{50}$ (µM)* | | | | | |
|---|---|---|---|---|---|---|
| Compound | TIBO' | 1073 | 1074 | G910 | G691 | HIV-2 |
| INK-3 | 0.11 | 0.90 | 0.19 | 0.20 | 0.12 | 0.21 |
| CP-51 | 0.10 | 0.19 | 0.20 | 0.64 | 0.33 | 0.32 |
| AZT | ND | 0.16 | 0.03 | >2.5 | >2.5 | ND |

*$EC_{50}$ values were calculated by the method of Chou and coworkers (Chou & Talalay, 1987; Johnson et al., 1989) as cited by Plantadosi et al. (1991).

Table 4. Evaluation of INK compounds for activity against matched pairs of AZT-sensitive and AZT-resistant HIV-1 clinical isolates

| | $EC_{50}$ (µM) | | |
|---|---|---|---|
| | AZT-sensitive strain | AZT-resistant strain | Fold increase |
| Compound | G-762 | G-691 | res./sen. |
| INK-14 | 0.10 | 2.34 | 23.4 |
| INK-15 | 0.06 | 0.08 | 1.3 |
| AZT | 0.003 | 2.20 | 673.3 |
| | H112-2 | G-910 | |
| INK-14 | 0.01 | 4.69 | 469 |
| INK-15 | 0.07 | 0.02 | <1 |
| AZT | 0.004 | 0.30 | 75 |

Assays were carried out using CEM-SS cells and the syncytial plaque assay.
Abbreviations; res: resistant; sen: sensitive

among all the compounds evaluated. In PBL cells, the $TC_{50}$ values were comparable to those from CEM-SS cells. However, the $EC_{50}$ values were consistently higher in PBL than in CEM-SS cells (Table 2). These results were most likely due to differences in sensitivity of prototype HIV-1 (strain H9IIIB) and a clinical isolate (strain H112-2) to the test compounds.

## Inhibition of HIV-2 and TIBO- and AZT-resistant HIV-1 strains as measured by syncytial plaque assay

Since selected PC lipid compounds (for example INK-3 and CP-51) have potent activity against prototype strains of HIV-1, we extended evaluation of their activity against HIV-2 and TIBO- and AZT-resistant HIV-1 clinical isolates. Results (Table 3) indicated that both INK-3 and CP-51 gave dose-dependent activity against HIV-2, with $EC_{50}$ values of 0.21 and 0.32 µM and against TIBO-resistant HIV-1 with $EC_{50}$ values of 0.11 and 0.10 µM, respectively. The $EC_{50}$ values for INK-3 and CP-51 against AZT-resistant HIV-1 clinical isolates (1073, 1074, G910, G691) ranged from 0.12 to 0.90 µM and 0.19 to 0.64 µM, respectively (Table 3). The $EC_{50}$ values for AZT against the same AZT-resistant HIV-1 clinical isolates ranged from 0.03 to >2.5 µM (Table 3). Note that the $EC_{50}$ for AZT against a sensitive prototype HIV-1 strain was 0.009 µM (Table 2). Other results indicated that the PC lipid INK-15 had no significant fold increase in $EC_{50}$ between the matched pairs of AZT-sensitive and AZT-resistant HIV-1 clinical isolates (Table 4). However, the lipid–AZT conjugate INK-14 and AZT alone showed a significant fold increase in the $EC_{50}$ ratio between the AZT-sensitive and AZT-resistant HIV-1 clinical isolates of 23.4, 469 and 673.3, 75, respectively. In summary, the PC lipids INK-3 and CP-51 have potent activity against HIV-2 and TIBO- and AZT-resistant HIV-1. There is no apparent cross-resistance between PC lipid and TIBO or AZT compounds. In contrast, the lipid–AZT conjugate (INK-14) did show some cross-resistance with AZT.

## Inhibition of fusogenic activity between persistently HIV-1-infected H9IIIB cells and uninfected CD4⁺ CEM-SS cells

Published data from our laboratories indicated that CP-51 significantly inhibited HIV-1-induced fusogenic activity (Krugner-Higby et al., 1995). In similar experiments (Table 5), results indicated that INK-3 also has significant dose-dependent activity against HIV-1-induced fusogenic activity. The $EC_{50}$ values were 0.08, 0.18 and >1.0 µM for INK-2, -3, and -1, respectively (Table 5). These results suggest that selected PC lipid compounds active against infectious HIV production (Table 2) also inhibited virus-induced fusogenic activity.

## Toxicity against mouse bone marrow cells as compared to AZT

Two independent experiments were designed to compare the relative toxicity of CP-51 and selected INK compounds with AZT. Results of these studies indicated that INK-2, -3 and -6 had a $TC_{50}$ value of >100 µg/ml (Table 6). In two experiments, the $TC_{50}$ values for CP-51 were 9.15 and 14.17 µg/ml compared to 0.142 and 0.259 µg/ml for AZT (Table 6). These data are interpreted to suggest that selected PC lipid compounds are significantly less cytotoxic than AZT to mouse bone marrow cells.

## Discussion

Most of our previously synthesized alkylamidopropyl phosphocholines and alkoxy- and alkylthioglycerol phosphocholines (PC lipids) were analogues of phosphatidylcholine and contained a long hydrocarbon chain length (C16–C18) at position 1 and a short chain length (C1–C2) at position 2 of the three carbon backbone. Many of these compounds exhibited potent anti-HIV-1 activity. The most promising analogue we have reported to date

Table 5. Effect of PC lipid compounds on fusogenic activity between persistently HIV-1-infected H9IIIB cells and uninfected CD4+ CEM-SS cells

| Compound | Concentration (μM) | Inhibition of fused cells (%) | $IC_{50}$ (μM) |
|---|---|---|---|
| INK-1 | 1.0 | 18 | |
| | 0.1 | 27 | |
| | 0.01 | 17 | >1.0 |
| INK-2 | 1.0 | 86 | |
| | 0.1 | 53 | |
| | 0.01 | 17 | 0.08 |
| INK-3 | 1.0 | 78 | |
| | 0.1 | 41 | |
| | 0.01 | 9 | 0.18 |

Persistently HIV-1-infected H9IIIB cells were treated with compound for 96 h and cocultured with uninfected CD4+ CEM-SS cells (ratio 1 : 100) in the presence of added compound for 48 h. The number of fused cells was counted relative to untreated control cells to determine the percentage inhibition of fusion and the $IC_{50}$. The $IC_{50}$ values were calculated by the method of Chou and coworkers (Chou & Talalay, 1987; Johnson *et al.*, 1989) as cited by Piantadosi et al. (1991).

Table 6. *In vitro* cytotoxicity of PC lipid compounds in CFU-GM assays

| Compound | $TC_{50}$ (μg/ml) | |
|---|---|---|
| | Experiment 1 | Experiment 2 |
| CP-51 | 9.15 | 14.17 |
| INK-2 | >100 | >100 |
| INK-3 | >100 | >100 |
| INK-6 | >100 | >100 |
| AZT | 0.142 | 0.259 |

In control cultures treated with PBS there were 86 and 54 c.f.u./plate in Experiments 1 and 2, respectively.

(Kucera *et al.*, 1990a) was 1-octadecanamido-2-ethoxypropyl-3-phosphocholine (CP-51). This amidoalkyl derivative exhibited an $EC_{50}$ of 0.11 μM and a SI of 130 (Table 2). Accordingly, we chose this analogue as our lead compound and proceeded to modify its structure to optimize its anti-HIV-1 inhibitory activity and/or SI. In addition, we wanted to ascertain whether a long hydrocarbon chain length at position 1 was essential for optimum anti-HIV-1 selectivity.

As seen from data in Table 2, numerous synthetic CP-51 analogues including INK-2, INK-3, INK-6 and INK-15 exhibited a higher SI than CP-51. The SI values for these analogues in CEM-SS cells were 184.0, 298.9, 191.4 and 389.0, respectively, whereas their $EC_{50}$ values were comparable to that of CP-51. These results demonstrated that a long hydrocarbon chain length of 16–18 carbons is not essential for optimum selectivity in this series of PC compounds. Notably, data presented in Table 2 suggested that a shorter hydrocarbon chain length (Table 1) at position 1 (10 or 12 carbons) and a longer chain at position 2 (12 or 8 carbons) as seen in INK-15 and INK-3, respectively, improved the SI up to threefold (with a concomitant decrease in cytotoxicity) compared with the 18 and 2 hydrocarbon chain lengths in CP-51.

Previously, we synthesized a series of alkyletherglycerol phosphocholines (Meyer *et al.*, 1991). Like the alkylamido phosphocholine CP-51, these alkylether compounds had a long hydrocarbon chain (C16–C18, oxy- or thioether) at position 1 and a short chain (methoxy or ethoxy) at position 2. The $EC_{50}$ values for these alkylether phosphocholines ranged from 0.3 to 1.4 μM and their SI were lower (4 to 69) compared to that of CP-51 (130). In view of the above data with the INK compounds, it was of interest to synthesize an alkylether analogue of INK-2 to determine whether the SI could be similarly improved in this series. This alkylether analogue, INK-4, with oxyether hydrocarbon chain lengths of 12 and 10 at the 1 and 2 positions, respectively, exhibited an $EC_{50}$ of 0.64 μM, a $TC_{50}$ of 75.7 μM, and a SI of 118.3. Notably, the $TC_{50}$ value is significantly different from that of any previously reported alkylether phosphocholine (3 to 19 μM; Meyer *et al.*, 1991), again demonstrating that such hydrodrocarbon chain length modifications will lead to a less cytotoxic compound. In summary, these data indicated that hydrocarbon chain lengths in the range of 10 to 12 at position 1 and 8 to 12 at position 2 should result in compounds with less cytotoxicity than those with 16 to 18 hydrocarbon chain lengths at position 1 and 1 to 2 at position 2.

Also, the $TC_{50}$ values from PBL cells were comparable to those from CEM-SS cells (Table 2). INK-13 and INK-14, with 8 and 10 carbons at position 1 and 7 carbons at position 2, respectively, were the least cytotoxic ($TC_{50}$ ≥100 μM) compounds in the series. At the present time, no specific experiments have been done to determine why INK-13 and INK-14 were the least cytotoxic in the series of compounds evaluated. In the case of the conjugate INK-14, the hypothesis is that the lipid component acts as a carrier for AZT. The lipid anchors the conjugate into the cell membrane and is metabolized to slowly release lower concentrations of AZT into cells compared to AZT treatment alone. The end result is reduced toxicity from AZT. Evidence in support of this hypothesis is the lower $TC_{50}$ and higher SI for INK-14 compared to AZT (Table 2).

In the case of the phospholipid–AZT conjugates, INK-14 [3′-azido-3′-deoxy-5′-(3-dodecylamido-2-octoxypropyl)-phosphothymidine] and CP-92 [3′-azido-3′-deoxy-5′-(3-octadecylamido-2-ethoxypropyl)-phosphothymidine] had comparable SI values [>1250 (Table 2) and 1793 (Piantadosi *et al.*, 1991), respectively]. Both compounds had a higher SI than AZT alone (411, Table 2) and were three- to fourfold more selective than AZT. In addition, the cytotoxicity was lower; the $TC_{50}$ for INK-14 was >100 μM in comparison to 3.7 μM for AZT.

The effects of lengthening the linkage between the phosphate ester and the quaternary ammonium functionality were also studied through the synthesis of INK-8, with three carbons separating the phosphate ester and the quaternary nitrogen. This modification increased the $EC_{50}$ and decreased the SI values (Table 2). At the present time, it is difficult to draw any definitive conclusion relating to such modification.

Based on these promising *in vitro* data, selected PC lipid and lipid–AZT conjugates were further evaluated. The results indicated that CP-51 and INK-3 also were active against HIV-2 and TIBO-resistant HIV-1 (Table 3). We also examined the effects of CP-51 and INK-3 against AZT-resistant HIV-1 clinical isolates (1073, 1074, G910, G691). As seen from the data in Table 3, both CP-51 and INK-3 were markedly active against the AZT-resistant mutants. Both the nucleoside analogue AZT and the non-nucleoside TIBO analogue R82150 (nevirapine; White *et al.*, 1991) are known to inhibit HIV-1 RT (De Clercq, 1995). However, unlike AZT, which can also inhibit HIV-2 RT activity, the non-nucleoside RT inhibitors do not inhibit HIV-2 RT activity. This difference in antiviral spectrum may be due to the non-nucleoside analogue binding at an allosteric site of RT, in contrast to the active site like AZT (De Clercq, 1995). From our present data, PC lipids are active against both TIBO- and AZT-resistant strains of HIV-1, suggesting that PC lipids do not target the same site(s) in the HIV replication cycle as compared to TIBO derivatives and AZT.

Using matched pairs of AZT-sensitive and AZT-resistant HIV-1 clinical isolates, results indicated that each pair of isolates tested was similarly inhibited by the PC lipid INK-15 (Table 4). These data are interpreted to suggest that PC lipids do not show cross-resistance with AZT. In contrast, these same pairs of clinical isolates did show increased resistance to the conjugate compound INK-14 and to AZT alone (Table 4), suggesting that the most active anti-HIV-1 component in the lipid–AZT conjugate compound is probably AZT.

Results in Table 5 indicate that PC lipids were also active in inhibiting fusogenic activity of HIV-1-infected cells with uninfected cells. Recent published evidence by other investigators (Feng *et al.*, 1997) indicated that HIV-1 enters cells via a co-receptor designated fusin (CXCR-4), a member of the G protein-coupled chemokine receptor family with seven member-spanning domains. McKnight *et al.* (1997) reported that a monoclonal antibody to fusin can block cell-to-cell fusion and cell-free virus infection of fusin-positive $CD4^+$ cells. Lipid analogues are known to accumulate at the cell plasma membrane (Storme *et al.*, 1985; van Blitterswijk *et al.*, 1987) and they serve as potent inhibitors of HIV-1-induced cell fusion (Krugner-Higby *et al.*, 1995). This inhibition was correlated with inhibition of gp160/gp120-specific monoclonal antibody with HIV-1 gp160/gp120 reactivity on the surface of HIV-1-infected and treated cells (Krugner-Higby *et al.*, 1995). It can be speculated that the presence of lipid analogues could cause steric hindrance of the $CD4^+$ receptor and fusin co-receptor in cell-to-cell fusion.

Published evidence indicates that the major antiviral effect of recombinant interferon α (rIFN-α) operates in the stages of virus assembly of gp120 and release (Hansen *et al.*, 1992; Smith *et al.*, 1991; Willey *et al.*, 1988). Syntheses of HIV-1-induced DNA, RNA and protein were minimally inhibited by rIFN α but virus particles released from the treated cells were 100- to 1000-fold less infectious, owing to an assembly defect in gp120 (Hansen *et al.*, 1992). Our published data (Krugner-Higby *et al.*, 1995) indicated that anti-HIV PC lipids do not affect HIV-1-induced protein synthesis and processing. However, assembly of progeny virus particles made in the presence of antiviral PC lipids was shifted from the plasma membrane to intracytoplasmic vacuoles (Kucera *et al.*, 1990b), and this shift in assembly site was associated with a profoundly reduced capacity of progeny virus particles to bind to $CD4^+$ cells (Krugner-Higby *et al.*, 1995). Also, we observed that exposure of fresh $CD4^+$ cells to equivalent amounts of RT activity associated with HIV-1 particles from 1 μM PC lipid-treated cells resulted in a 40 to 68% inhibition in subsequent HIV-1 replication compared to HIV-1 particles from untreated control cells (LS Kucera, N Iyer, SL Morris-Natschke, SY Chen, F Gumus, K Ishaq & DBJ Herrmann, unpublished results). Data from Western blot analysis of virus particles from PC lipid-treated cells indicated that gp120 produced in infected cells was also present in virus particles recovered from PC lipid-treated cell supernatants (Krugner-Higby *et al.*, 1995). Incubation of PC lipid with preformed virions did not produce a virucidal effect (LS Kucera, N Iyer, SL Morris-Natschke, SY Chen, F Gumus, K Ishaq & DBJ Herrmann, unpublished results). Krugner-Higby *et al.* (1995) showed that HIV particles made in the presence of phospholipids had a reduced capacity to bind to $CD4^+$ cells. Taken together, our data suggest that the reduced capacity of progeny virus from PC lipid-treated cells to infect and replicate in fresh $CD4^+$ cells could be associated with an alteration in the assembly of gp120 on the surface of progeny virus particles. Thus, PC lipids may share a property in common with rIFN-α by operating at the stage of HIV gp120 assembly and release.

In summary, results from chemical synthesis and evaluation of compounds for anti-HIV-1 activity in this present investigation indicated that compounds with hydrocarbon chain lengths of 10 to 12 at position 1 and 8 to 12 at position 2 of a three carbon backbone have high SI against HIV-1 replication. PC lipid analogues have potent and

selective activity against HIV-1 and HIV-2 as well as against TIBO- and AZT-resistant strains of HIV-1 and virus-induced cell fusion. In view of the markedly reduced toxicity of PC lipid analogues against bone marrow cell growth *in vitro* compared to AZT, PC lipid analogues offer an innovative approach toward development of novel strategies for the treatment of HIV infections and AIDS.

## References

Chou T-C & Talalay P (1987) Applications of the median-effect principle for the assessment of low-dose risk of carcinogens and for the quantitation of synergism and antagonism of chemotherapeutic agents. In *New Avenues in Developmental Cancer Chemotherapy*, vol. 8, pp. 37–64. Bristol-Myers Cancer Symposia. Edited by K Harrap & T Connors. New York: Academic Press.

De Clercq E (1995) Toward improved anti-HIV chemotherapy: therapeutic strategies for intervention with HIV infections. *Journal of Medicinal Chemistry* 38:2491–2517.

Feng Y, Broder CC, Kennedy E & Berger EA (1997) HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 272:872–877.

Hansen BD, Nara PL, Maheshwari RK, Sidhu GS, Bernbaum JG, Hoekzema D, Meltzer MS & Gendelman HE (1992) Loss of infectivity by progeny virus from alpha interferon-treated human immunodeficiency virus type 1-infected T cells is associated with defective assembly of envelope gp120. *Journal of Virology* 66:7543–7548.

Hansen WJ, Murari R, Wedmid Y & Baumann WJ (1982) An improved procedure for the synthesis of choline phospholipids via 2-bromoethyl dichlorophosphate. *Lipids* 17:453–459.

Johnson VA, Walker BD, Barlow MA, Paradis TJ, Chou T-C & Hirsch MS (1989) Synergistic inhibition of human immunodeficiency virus type 1 and type 2 replication in vitro by castanospermine and 3′-azido-3′-deoxythymidine. *Antimicrobial Agents and Chemotherapy* 33:53–57.

Krugner-Higby L, Goff D, Edwards T, Iyer N, Neufeld J, Kute T, Morris-Natschke S, Ishaq K, Piantadosi C & Kucera LS (1995). Membrane-interactive phospholipids inhibit HIV type 1-induced cell fusion and surface gp160/gp120 binding to monoclonal antibody. *AIDS Research and Human Retroviruses* 11:705–712.

Kucera LS, Iyer N, Leake E, Raben A, Modest EJ, Daniel LW & Piantadosi C (1990a) Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation. *AIDS Research and Human Retroviruses* 6:491–501.

Kucera LS, Iyer N, Leake E, Raben A, Daniel L, Modest EJ & Piantadosi C (1990b) Effect of membrane-active ether lipid (EL) analogs on human immunodeficiency virus production measured by plaque assay. *Annals of the New York Academy of Sciences* 616:545–548.

McKnight A, Wilkinson D, Simmons G, Talbot S, Picard L, Ahuja M, March M, Hoxie JA & Clapham PR (1997) Inhibition of human immunodeficiency virus fusion by a monoclonal antibody to a co-receptor (CXCR4) is both cell type and virus strain dependent. *Journal of Virology* 71:1692–1696.

Meyer KL, Marasco CJ Jr, Morris-Natschke SL, Ishaq KS & Piantadosi C (1991) *In vitro* evaluation of phosphocholine and quaternary ammonium containing lipids as novel anti-HIV agents. *Journal of Medicinal Chemistry* 34:1377–1383.

Morris-Natschke SL, Gumus F, Marasco CJ Jr, Meyer KL, Marx M, Piantadosi C, Layne MD & Modest EJ (1993) Synthesis of phosphocholine and quaternary amine ether lipids and evaluation of in vitro antineoplastic activity. *Journal of Medicinal Chemistry* 36:2018–2025.

Piantadosi C, Marasco CJ Jr, Morris-Natschke SL, Meyer KL, Gümüs F, Surles JR, Ishaq KS, Kucera LS, Iyer N, Wallen CA, Piantadosi S & Modest EJ (1991) Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV-1 activity. *Journal of Medicinal Chemistry* 34:1408–1414.

Ryu EK & MacCoss M (1979) Modification of the Dittmer–Lester reagent for the detection of phospholipid derivatives on thin-layer chromatograms. *Journal of Lipid Research* 20:561–563.

Smith MS, Thresher RJ & Pagano JS (1991) Inhibition of human immunodeficiency virus type 1 morphogenesis in T cells by alpha interferon. *Antimicrobial Agents & Chemotherapy* 35:62–67.

Storme GA, Berdel WE, van Blitterswijk WJ, Bruyneel EA, DeBruyne GK & Mareel MM (1985) Anti-invasive effect of racemic 1-O-octadecyl-2-O-methylglycero-3-phosphocholine on M04 mouse fibrosarcoma cells in-vitro. *Cancer Research* 45:351–357.

van Blitterswijk WJ, Hilkmann H & Storme GA (1987) Accumulation of an alkyl lysophospholipid in tumor cell membranes affects membrane fluidity and tumor cell invasion. *Lipids* 22:820–823.

White EL, Buckheit RW Jr, Ross IJ, Germany JM, Andries K, Pauwels R, Janssen PAJ, Shannon WM and Chirigos MA (1991) A TIBO derivative, R82913, is a potent inhibitor of HIV-1 reverse transcriptase with heteropolymer templates. *Antiviral Research* 16:257–266.

Willey RL, Bonifacino JS, Potts BJ, Martin MA & Klausner RD (1988) Biogenesis, cleavage, and degradation of the human immunodeficiency virus type 1 envelope glycoprotein gp160. *Proceedings of the National Academy of Sciences, USA* 85:9580–9584.

Received 18 August 1997; accepted 4 November 1997